Coulochem® III

Reference Manual

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NOTICES:

This system is covered by a limited warranty. A copy of the warranty is included with this manual. The analyst is required to perform routine maintenance as described herein on a periodic basis to keep the warranty in effect.

All information in this manual is subject to change without notice and does not represent a commitment on the part of ESA, Inc.

The Coulochem III and various components in the system are covered by the following patents: US: 4,233,031; RE32,920; 4,404,065; 4,497,199; 4,511,659; 4,552,013; 4,753,714; 4,804,455; 4,863,873; 4,976,994; 5,104,639. Canada: 1138043 (1982); 1139841 (1983); 1167277 (1984); 1167526 (1984); 1195383 (1985); 1238362 (1988); 1251515 (1989); 1271811 (1990). Japan: 1536120; 1827931; 2018280; 2059320; 2072937. France: 2422948; 0223532; 0227281; 0033188; 0567564. Germany: P3681691.4; P3686030.1; 3174440; 0567564. Italy: 0223532; 0227281; 0033188. EPO: 0122009. UK: 2012435B; 0223532; 0227281; 0033188; 0567564.

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This warranty shall be governed by, and construed and enforced in accordance with the substantive laws of the Commonwealth of Massachusetts.

This warranty shall be non-transferable and shall run to the benefit of the original purchaser only.

WARNINGS AND SAFETY PRECAUTIONS

The ESA Coulochem® III is an electrochemical detector system for high performance liquid chromatography. It can be used to determine the level of a large number of electroactive compounds in a broad variety of samples including those of biological origin. The following precautions should be followed to minimize the possibility of personal injury and/or damage to property while using the instrument.

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1) **Maintain a Well-Ventilated Laboratory.**
The mobile phase typically contains a volatile organic solvent. Ensure that the laboratory is well ventilated so that a buildup of vaporized solvent cannot occur. The eluent should be collected in a container that minimizes the escape of waste solvent into the atmosphere.

2) **Avoid Open Flames and Sparks.**
The mobile phase typically contains a flammable organic solvent. Do not use an open flame in the laboratory and do not install any equipment that can cause sparks in the same room as the instrument.

3) **The Instrument must be Plugged into a Grounded Power Line.**
Ensure that all parts of the system are properly grounded. It is strongly recommended that all parts of the system are connected to a common ground. Do **not** attempt to bypass the earth ground connection. A serious shock hazard could result.

4) **Treat all Samples and Mobile Phases as if they are Capable of Containing Hazardous Substances or Transmitting Disease.**
The sample and/or mobile phase may contain compounds that may present a hazard to the operator of the system. Take all precautions to ensure that the mobile phase does not come into contact with the skin or eyes. A sink and eyewash should be located in the laboratory. In the event of an accident wash the affected part with copious quantities of water and seek medical assistance. If you are analyzing biological/clinical samples, treat them in accordance with the infectious disease control program of your institution.

5) **Ensure that the Unit has been Disconnected from the Line before Removing the Cover.**
Potentially hazardous currents and voltages may be present inside the unit.

6) **Wear Protective Eyewear.**
Solvents and other chemicals can damage eyes. Install a sink as close as possible to the module. If any solvents or chemicals splash on the skin or eyes, immediately rinse the affected parts in the sink. Obtain medical help as needed.

7) Monitor and Maintain Proper Pressure in Tubing and Devices used in the Coulochem Organizer.
The maximum recommended pressure rating of tubing and devices used in the Coulochem III Organizer is 275 bar when using PEEK™ tubing. The maximum pressure limit used also depends on the specific device(s) used in the organizer. Refer to the device's manufacturers for their maximum rating.

8) Use CE Approved HPLC Pump(s) or Solvent Delivery Device(s).
The pump(s) used with the Coulochem III Organizers must be CE approved and contain a properly installed and maintained pressure safety device and/or pressure sensor that conforms to the requirement of ISO 4126-1.

9) Avoid Use of Some Solvents with PEEK Tubing.
While PEEK tubing has excellent chemical resistance to most common organic solvents, it is attacked by concentrated nitric acid and sulfuric acid, and tends to swell (and weaken) in solutions with high concentrations of chloroform, dimethylsulfoxide, tetrahydrofuran and similar solvents. If these solvents must be used in high concentrations, the use of stainless steel may be appropriate.

10) Use the Instrument in a Proper Manner.
Do not use the detector and its accessories in a manner not specified by ESA. Otherwise the safety protection provided by the equipment may be impaired.

11) Replace the Fuse Only with One of the Same Type and Rating.
For continued protection against fire, replace the line fuse only with a fuse of the specified type and rating.

12) Do not Touch Heated Surfaces Inside the Thermal Organizer.
If the Thermal Organizer temperature is elevated, do not touch heated components.

13) ESA Electrochemical Cells should not be Maintained Above 45°C.
If the temperature of the Thermal Organizer is kept above 45°C (i.e., the separation is effected at an elevated temperature), make sure that the cells are placed outside the organizer as elevated cell temperatures will dramatically shorten cell lifetimes.

WARNING: Before connecting this detector to any MS Unit, the ESA Hi-Voltage Decoupling Union Kit (P/N 70-5974) must be properly installed (See Appendix E).

SAFETY/OPERATING SYMBOLS

The following symbols appearing on the unit or in the manual are defined as follows:

This symbol on the instrument indicates that the user should refer to the operating manual before attempting to connect the power/interface cables and operate the system.

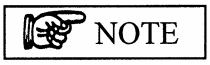
This symbol on the instrument states that high voltage may be present when panels/covers are removed. Any adjustment, maintenance, and repair of the opened apparatus under voltage should be avoided as far as possible and, if inevitable, must be carried out only by a skilled person who is aware of the hazard involved.

This symbol on the instrument states that elevated temperatures may be present in the system. The user should take care that the internal components are not touched when the temperature is elevated.

This symbol on the back of the instrument indicates a ground terminal.

The blocked WARNING statement used throughout the manual presents dangers that might result in personal injury.

The blocked CAUTION statement used throughout the manual presents hazards on conditions that could cause damage to the instrument or the reporting of erroneous results.



The blocked NOTE statement used throughout the manual highlights important information about the detector and its use.

Failure to follow these statements may invalidate the warranty.

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IMPORTANT OPERATING CONSIDERATIONS

A detailed discussion of the operation of the detector is presented in Chapters 5-7. This section describes some considerations that are especially important for optimizing performance of the system.

A) Ensure that In-line Filters are used before each Electrochemical Cell in the System

ESA cells with model number 501X (e.g., Model 5010A Standard Analytical Cell) and Model Number 502X (e.g., Model 5020 Guard Cell) contain porous graphite electrodes. These electrodes have a very large surface area to ensure a coulometric response. The graphite electrode can act as an efficient filter for small particles, such as silica fines from the column and particulate matter in the sample. These particles tend to clog the electrode and impede the flow of the mobile phase. To avoid clogging the electrode, **an in-line filter containing a filter element MUST precede each electrochemical cell in the system.** If the system uses a guard cell and an analytical cell, it will be necessary to use two in-line filters.

In addition, it is strongly recommended that the aqueous portion of the mobile phase and all samples are filtered through a 0.22 micron filter.

The label illustrated below is placed on ESA cells as a reminder that the use of in-line filters is essential.

THE ESA, INC. WARRANTY DOES NOT
COVER ELECTRODES WHICH HAVE FAILED
DUE TO IMPEDED FLOW.

B) Real-Time Clock Battery Backup

The clock circuitry contains a battery that is an integrated part of the system. This battery is potted and sealed inside the chip and is not replaceable. The life of the battery is specified with a minimum lifetime of 10 years.

C) Use a Line Voltage Conditioning Device

If the input power to the Coulochem III is noisy (i.e., fluctuations in the line voltage from the power source such as spikes, sags, dropouts, brownouts, etc.) interruptions in the use of the instrument may be observed. Interruptions usually cause the unit to "reset"; this will stop the unit, remove the potential from the cells and return the display to the initial screen or "greeting" screen.

On rare occasions, the display may "lock up" or present nonsense information. If a Coulochem III has a locked up display or shows nonsense information:

1. Turn off the power to the unit.
2. Wait about a minute and then turn the power back on.
3. Continue to use the unit as desired.

While the Coulochem III includes an in-line filter, excessive fluctuations and brownouts may create problems. If line voltage fluctuations or brownouts are a common occurrence in your laboratory or if you would simply like to ensure that power fluctuations do not lead to lock ups, loss of analytical methods or default storage methods; the use of a line voltage conditioner or power surge protector *with power sag* protection is recommended. These devices can usually be obtained from sources that carry computer supplies.

ESA recommends that the line voltage conditioning device meets or exceeds the following specifications:

a) Response time of 1 ns or less.

b) Dropout voltage of about 75% or greater than the stated line voltage (e.g., in the USA the line voltage is 120 V so that a dropout voltage of 85 V or higher would be acceptable).

c) Clamping level of 130 V for stated line voltages of 120 V or less and clamping level of 275 V for stated line voltages of 230 V or higher.

If you have any questions concerning this, please contact the ESA Service Department or its Representative.

CAUTIONS

This guide contains important information about factors that can effect the accuracy of test results and should be reviewed before the detector is used. This is especially important if the Coulochem® III is to be used in a clinical setting as part of a system and method for *in vitro* diagnostic testing.

Inaccurate results *might* occur if the Coulochem III detector is not used according to the instructions presented in the *Coulochem III (50W) User's Guide* and *Reference Manual*. The following are particularly important:

- Environment of the Coulochem III detector
- Analysis of the resulting chromatograms

1 Operating Environment

Erroneous results *might* occur as the result of the following environmental conditions:

- Power Line Fluctuations (surge or sag)
- Improper Line Voltage
- Strong Radio Frequency Interference (RFI)
- Strong Electrostatic Discharge (ESD)
- Large Temperature Fluctuations
- Extreme Temperature

The use of an Uninterruptible Power Supply (UPS) system is recommended to prevent the potential deleterious effects of power line fluctuations, interruptions or improper line voltage. This is especially important if the AC power to your facility is unstable and/or susceptible to brown-outs, spikes, surges, sags, dropouts and other interruptions. If a UPS is used, ESA recommends that it meets or exceeds the following specifications:

- Transfer time of 10 milliseconds or less.

- Dropout voltage of about 75% or greater than the stated line voltage (e.g., in the USA the line voltage is 120 V, so that a dropout voltage of 85 V or higher would be acceptable).

- Clamping level of 130 V for stated line voltages of 120 V or less and clamping level of 275 V for stated line voltages of 230 V or higher.

- Capacity of 500 VA or greater (this should allow the detector to run for several hours in the event of a total power failure).

☞ NOTE: A larger capacity for the UPS device is needed if the entire HPLC system is to be covered by this UPS device. In this situation, the total power consumption of your entire HPLC system must be determined and the desired period of time that the system should operate after the power interruption must be determined to calculate the proper capacity of the UPS system.

At the very least, a passive line conditioner (such as a surge protector, line filter, etc.) is recommended for the detector. In addition, we strongly recommend that the Coulochem detector *not* share a circuit with other electrical equipment that draws significant power from the AC line. This equipment includes items such as fume hoods, refrigerators, ovens, centrifuges, refrigerated cooling systems, vacuum pumps, etc.

During installation of the detector or if the detector is moved, make sure that the voltage option of the power input receptacle on the back of the detector is set for the same voltage of the AC circuit to which it will be connected. The likely outcome of various power fluctuations is the introduction of a disturbance or artifact in the chromatogram. These should be readily discernable when the operator reviews the chromatogram and can be dealt with as applicable.

Strong RFI may also cause disturbances or artifacts in the chromatogram or with the operation of the detector. The ESA Coulochem III has been designed to meet various certification agencies stringent requirements for susceptibility to RFI. However, it is still possible that a strong emitter of RFI could result in interference with the Coulochem III detector. Therefore, the detector should not be placed close to other instruments or machinery that could emit excessive RFI or magnetic fields such as refrigerators, fume hoods, radio transmitter antennae, NMR instruments, etc.

In addition, the Coulochem III has been designed to be immune to electrostatic discharge (ESD). However, strong electrostatic discharges to the instrument could cause a disturbance or artifact to the baseline or could interfere with the operation of the detector. ESA strongly recommends that the detector is properly grounded to a good earth ground. This should result in most ESD events being harmlessly conducted to ground and thus avoiding changes or damage to the sensitive electronics. In addition, we recommend that the operator take steps to reduce the generation of static electricity in the vicinity of the detector. Measures to reduce the chance or severity of ESD include:

- Increasing the relative humidity (especially during the winter)
- Ensuring that the operator touches a good earth ground (e.g., a metal water pipe) just prior to touching the detector
- Installing antistatic mats in front of the detector
- Avoiding the use of carpeting near the detector
- Low or antistatic lab coats

Large temperature fluctuations can *potentially* lead to baseline disturbances or other interference with the operation of the Coulochem III detector. Similarly, operating the detector at temperatures outside of its intended operating temperature range (10°-35°C or 50°-95°F) - *might* lead to artifacts or other instrumental interference. Therefore, the temperature of the laboratory in which the Coulochem III is used should be controlled. The laboratory temperature should be within the specified operating temperature range and temperature fluctuations should be kept at a minimum. Avoid placing the instrument in drafts (such as near an air conditioning or heating duct, the heat output of a refrigerator or an open window) and in direct sunlight.

On rare occasions, the display and/or the detector may "lock up" or present nonsense if one of the above-described situations occurs. If the Coulochem III has a locked up display or shows nonsense information:

1. Turn off the power to the unit.
2. Wait about a minute.
3. Turn the power back on.
4. Continue to use the detector as desired.

2 Analysis of Chromatograms

In addition to the environmental considerations described above, a variety of operator actions may lead to an analytical error. Typical examples of this include incorrect preparation of the mobile phase, standards or sample; injection of an incorrect sample volume, incorrect instrumental parameters (e.g., the flow rate or the detector potential) or misidentification of the sample.

Most of the operator errors and the errors due to environmental situations can be detected by careful review of each and every chromatogram before the results are reported. The operator should examine each chromatogram for baseline disturbances and artifacts. In addition, the operator should examine each chromatogram of the samples for proper retention time of important peaks, for interfering peaks, expected peak shape and for chromatogram integrity. All chromatograms of controls and standards should be similarly scrutinized. If a questionable result is obtained, the sample should be rerun. It is also advisable in questionable cases to rerun controls (and standards if necessary). If the controls and standards do not provide chromatograms that match previously collected chromatograms for these samples, then the entire HPLC system should be examined for problems. For troubleshooting information, see Chapter 8 of this Manual.

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Table of Contents

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1 Introduction

1.1 Overview

The Coulochem® III (50W) Detector is designed for the detection of electroactive species in the eluent from a high performance liquid chromatograph. Documentation for the detector includes:

- ***Coulochem III (50W) Reference Manual*** (Part Number 70-6501) - provides a detailed discussion on the care and use of the detector, including method development, installation, maintenance and advanced topics such as the use of Pulse, Scan and Redox Modes (Pulse and Scan Modes require the *Pulse/Scan Potentiostat Board*).
- ***Coulochem III (50W) User's Guide Manual*** (Part Number 70-6502) - designed to provide sufficient information to allow the analyst to collect analytical data in DC mode (DC Mode requires the use of the *DC Potentiostat Board*).

The analyst who desires a complete description of the detector and its capabilities should refer to both manuals.

In general, the *Reference Manual* will be of primary interest to those individuals who will install and have overall responsibility for the detector (maintenance and troubleshooting) as well as those who are interested in method development.

1.2 Contents of the Coulochem III (50W) Reference Manual

The *Coulochem III (50W) Reference Manual* includes the following material:

- ***Setting Up the Detector in the Laboratory*** (Chapter 2) **-** describes the required laboratory environment, installing the detector in the laboratory, making electrical connections, and performing a test protocol to determine if the detector is functioning in an acceptable manner.
- ***Installing the Detector in an HPLC System*** (Chapter 3) - describes how the detector is connected to the HPLC system and includes a step by step procedure to assist the installer. In addition, a number of experiments are included to check overall system operation.
- ***Theory of Operation*** (Chapter 4) - discusses the principles of electrochemical detection in HPLC and includes a discussion of coulometric and amperometric detection modes. In addition, this chapter describes how electrochemical detection provides superb sensitivity and selectivity.

- ***The Coulometric Cell in Electrochemical Detection*** (Chapter 5) - describes the characteristics of the various coulometric cells that are used with the Coulochem III detector and explains the benefits of coulometric detection. In addition, this chapter describes maintenance and cleaning of coulometric cells.

- ***The Amperometric Cell in Electrochemical Detection*** (Chapter 6) - describes the characteristics of the amperometric cells that are used with the Coulochem III detector and explains the benefits of amperometric detection. In addition, this chapter describes maintenance and cleaning of amperometric cells.

- ***Maintenance Activities and Replacing Components*** (Chapter 7) **-** presents a discussion about a variety of activities that should be performed on a routine basis to optimize system performance.

- ***Troubleshooting*** (Chapter 8) **-** includes a protocol that the user should follow to determine the cause of difficulties.

- ***Programming the Detector for DC Timeline Operation*** (Chapter 9) - describes how the timeline feature can be used to schedule a variety of activities at the start of the run or at user specified times during a DC run.

- ***Screen Mode Operation*** (Chapter 10) - describes the use of Screen mode to eliminate possible interferents in an analytical procedure via a screening electrode.

- ***Redox Mode Operation*** (Chapter 11) **-** explains how the concentration of an analyte can be determined by electrochemically reducing/oxidizing a compound of interest.

- ***Pulse Mode Operation*** (Chapter 12) - discusses the use of pulse mode to condition the surface of the electrode during the analysis. This mode of operation is commonly used when the electrochemical process generates materials that foul the cell surface during analysis.

- ***Scan Mode Operation*** (Chapter 13) **-** describes how the potential can be changed during a run and how a cyclic voltammogram can be obtained.

- ***Coulochem III Organizer Modules*** (Chapter 14) **-** discusses the installation and use of the Organizer Module and Thermal Organizer Module options.

A series of appendices that provide detector specifications and information about maintaining cell performance are included in the manual.

1.3 Contents of the Coulochem III (50W) User's Guide Manual

The *Coulochem III (50W) User's Guide Manual* contains the following material:

- ***Introduction*** (Chapter 1) **-** describes the use of the detector in an HPLC system and the modes of operation. In addition, it describes the components in the detector unit and the various cells that are available.

- ***The User Interaction Program*** (Chapter 2) - discuses the general layout of the user interaction program and shows how the various operating modes are accessed. In addition, this chapter describes how a DC Mode method is established and executed.

- ***Operation of the Detector in an HPLC System*** (Chapter 3) - considers a broad variety of topics such as the selection of operating parameters, startup procedures, shutdown procedures and other topics that relate to the routine use of the detector.

- ***Mobile Phase Considerations*** (Chapter 4) **-** describes how the mobile phase for electrochemical detection should be generated and stored.

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2 Setting Up the Detector in the Laboratory

2.1 Introduction

This chapter describes how the detector should be set up in the laboratory and includes protocols to determine if it is functioning in an acceptable manner. It includes the following:

- Unpacking the detector (Section 2.2)
- Power requirements (Section 2.3)
- Locating the detector in the laboratory (Section 2.4)
- Electrical connections to other devices (Section 2.5)
- Test protocols (Section 2.6)
- Coulochem Worksheets (Section 2.7)

After it has been determined that the detector is working in an acceptable manner, it should be incorporated into the HPLC system as described in Chapter 3.

2.2 Unpacking the Detector

Carefully unpack your shipment and inspect the contents to verify receipt of all components. A *Customer Inventory Checklist*, which lists the parts shipped with the unit, is included in the front pocket of the manual. The Coulochem® III is shipped in a single carton containing the detector unit, cell(s) and accessory kits. The DC Potentiostat Module and/or the Pulse/Scan Module are installed in the detector unit. If the *Coulochem III Organizer* accessory or *Coulochem III Thermal Organizer* accessory is included, it will be shipped in a separate carton.

Carefully inspect the shipping carton and all components. If there is any damage to the carton or to any components, contact both the shipping agent and ESA (or its representative) immediately. If any parts are missing, call ESA's customer service department and indicate the missing items via the Part Numbers.

⚠ WARNING: If there is any evidence that the Coulochem III Detector unit has been damaged in shipping, do not plug the unit into the line. Contact ESA [(800) 275-0102] or its representative for advice.

The shipping carton should be retained as it can be used if it becomes necessary to transport the detector.

2.3 Power Requirements

⚠ WARNING: The Coulochem III Detector uses a three-prong power cord that includes a ground wire. The unit must be connected to a properly grounded three-prong power outlet to ensure safety and proper operation. If there is any doubt about the power supply, a qualified electrician should be contacted to ensure a properly operating and properly grounded power outlet.

The input power is 100-240 VAC 50/60Hz, no input configuration of power is necessary.

The 120 VAC power cable consists of a 3-prong receptacle for attachment to the power inlet on the back of the Detector unit and a three prong plug for connection to a standard U.S. grounded output.

The 100/230/240 VAC power cable consists of a 3-prong receptacle for attachment to the power inlet on the back of the Detector unit. The other end of the cable has three color-coded wires that are used to attach to the appropriate plug. The color-coding of the wires meets ISO and VDE conventions as follows:

Earth Ground	Green with Yellow Stripe
Neutral	Blue
Line	Brown

⚠ WARNING: The power plug should be installed by a qualified electrician and should be an approved plug (e.g., CE, TUV).

The power consumption of the unit is approximately 26 VA for units without the Thermal Organizer power supply and 100 VA max for systems with the Thermal Organizer power supply.

The detector should be connected to an electrical line that shares a common ground with other components of the chromatographic system (e.g., computers, recorders, the HPLC system controller, pump, autosampler, etc.). This will avoid "ground loops" which can create erratic results (e.g., varying background, high noise, etc.).

Use a power strip to plug all HPLC components into a common ground, if necessary. Although the detector contains a built-in line filter to reduce interference at any input voltage, connection to an electrical line which also serves units with a large power drain or which may be subject to power surges (typical systems of this type include centrifuges, ovens, refrigerators and fume hoods) is not recommended.

In addition, a surge suppressor or an uninterrupted power supply (UPS) should be used (specifications for the line voltage conditioning device are presented on page viii). Surge suppressors or uninterrupted power supplies designed for personal computers are suitable.

The Coulochem III Electrochemical Detector meets the requirements of Underwriters Laboratories (UL), Canadian Standards Association (CSA) and European Certification (CE).

2.4 Locating the Detector in the Laboratory

NOTE: The detector should be placed in a position so that distance between the end of the column and the flow cell of the detector can be minimized. This will reduce post column band broadening effects and optimize chromatographic resolution.

The detector should be placed in an area that is free from drafts or significant temperature changes. Avoid placing the detector near air conditioning vents, windows, ovens, etc.

The detector and associated HPLC system should be placed on a sturdy laboratory bench or table that provides access to all components and provides sufficient working space.

2.5 Electrical Connections to Other Devices

2.5.1 Description of the Rear Panel of the Control Module

All electrical connections are made via the rear panel of the detector. The rear panel is shown in Figure 2-1.

Figure 2-1: Rear Panel - Coulochem III Electrochemical Detector with both DC Potentiostat and Pulse/Scan Modules Installed

Cables provided by ESA are described in Table 2-1 and the simulator test loads provided by ESA for the appropriate configuration are described in Table 2-2. The appropriate cell cables and simulator test loads will be provided with the system and will be listed on the system configuration sheet provided with the system.

The Power Supply module (right-most panel) that is normally provided for systems which include the Coulochem III Organizer Module (or do not include an Organizer Module) does not have a jack for the Thermal Organizer Module option.

Table 2-1: Cables for the Coulochem III

Part Number	Description	Where Used
70-4769	Cable, Coulochem III, Dual Channel	Analytical Cell, DC Potentiostat (2 Channel Cell, e.g., Model 5011)
55-0173	Cable, Guard Cell	Guard Cell Potentiostat
70-4770	Cable, Coulochem III, Single Channel	Analytical Cell, DC Potentiostat (1 Channel Cell, e.g., Model 5041)
55-0179	Cable, Single Channel Analytical Cell	Pulse/Scan Potentiostat
70-1776	BNC to Bare Wire	DC/Pulse/Scan Mode XY Recorder Signal Out
70-4850	I/O, Bare Wire to Bare Wire	Logic Module, I/O Connections
70-1743	Cable, RS232	Coulochem III to Personal Computer
70-5713	Cable, USB	Coulochem III to Personal Computer
70-5595A	Cable, Interface Thermal Organizer (50 Watt)	Thermal Organizer to Power Input Module and Logic Module

Table 2-2: Simulator Test Loads

Part Number	Description	Where Used
70-4756	Cell Simulator Test Load, Single Channel	End of Single Channel Cables (Part Number 70-4770 and Part Number 55-0179)
70-4755	Cell Simulator Test Load, Dual Channel	DC Potentiostat on Detector
70-1790	Cell Simulator Test Load	End of Dual Channel Cable (Part Number 70-4769) and on Pulse/Scan Potentiostat on Detector
55-0172	Guard Cell Simulator Test Cell	Guard Cell Potentiostat and Cable; End of Single Channel Cable (Part Number 55-0179)

The terminals on the rear panel of the Coulochem III are used as follows:

- ***Analytical Cells*** - Analytical cells are connected to potentiostat modules in the detector using the appropriate cell cable (see Table 2-1). The 15-pin D type subminiature male connector is inserted into the DC potentiostat module.

- ***Recording Devices*** *(Integrators and Recorders)* - The recording device is connected to the potentiostat module using the BNC Male to Bare Wire Cable (Part Number 70-1776). Two cables are required for DC operation with a dual channel cell. A BNC Cable is inserted into a BNC connector labeled DC SIGNAL OUT 1 or DC SIGNAL OUT 2. Each cell output can be connected to a recording device.

  The BNC Male to Bare Wire Cable (Part Number 70-1776) can be used to connect the BNC socket to the recorder or other device that requires a bare wire connection.

  A BNC adapter (Part Number 70-0219) can be used to connect stripped wires, spade lugs or male banana plugs to a recording device with a female BNC connector on its input.

- ***Computers and External Terminals*** - The Coulochem III can be interfaced to an ESA Data Station, a personal computer or an external terminal via an RS232 Cable (Part Number 70-1743). The ESA Data Station includes a personal computer, printer, an RS232 Cable and software to provide complete control of the detector. The RS232 cable is connected to the detector via the 9-pin socket labeled *RS232 Remote* on the logic module and the serial port of the data station.

  When the detector is under remote control, some of the keys (**Autozero,** and **Cells On/Off**) are functional. To re-establish local control, press **Escape** or enter **R;0** (or the equivalent command) from the external device.

- ***Thermal Organizer Module*** - If a Thermal Organizer Module is installed, the Y cable (Part Number 70-5595A) is connected to the Thermal Organizer Control on the Logic Module and the Thermal Organizer Power connectors on the Power Supply Module on the rear panel of the detector and to the Thermal Organizer at the back and under the Chassis Plate.

- ***Guard Cell or Conditioning Cell*** - A Guard Cell or Conditioning Cell can be connected to the detector using the Guard Cell cable (Part Number 55-0173) that terminates in a male 9-pin D connector. The connector is on the DC PSTAT module and is labeled GUARD CELL. The cell cable must be connected before the Coulochem III is powered up for the appropriate menu options to be displayed.

- ***X-Y Recorder*** - An X-Y recorder is recommended for Scan mode. The X-Y recorder connector (BNC output) is used to install the cable for the X-axis. This output provides the recorder with the potential that is applied to the electrode during the scan. If desired, the potential can be divided by 1, 2, 5 or 10 to optimize the X-scale of the recorder.

  The Y-axis of the recorder is connected to the signal out BNC of the Pulse/Scan potentiostat to monitor the current.

- ***Autosamplers and other External Automated Devices*** - The Input/Output Connections block contains eight connections for interfacing the detector to external devices such as autosamplers, data stations, integrators, pumps, valves, etc., which can automate the operation of these devices. The inputs are TTL and the outputs are true relays. The two green terminals blocks must be installed before the inputs/outputs can be used. Make sure they are oriented and aligned properly before pushing or snapping them into place.

  OUTPUTS - The terminals labeled CC1 to CC5 provide a contact closure that can be used to start an autosampler, integrator, pump gradient, change a valve position, etc. These are used only when the timeline function is activated (see Chapter 9 and 12). Each of the terminals acts in a similar fashion, the labeled designations are simply provided for the convenience of the user.

  When the output contact closures are used, there is no polarity (plus "+" or minus "-"); however there is a third wire (the ground wire on Part Number 70-4850 cable), which should be attached to ground (GND) of the Coulochem III detector.

  INPUTS - The connector labeled STRT is used when a method involving the timeline program is to be started from an external device such as an autosampler. A contact closure or shorting of the terminals (a signal of at least 0.5 second) is required from the external device.

  It is strongly recommended that a true contact closure or relay closure be used for the inputs on the I/O connections block (CELL OFF, AZ and STRT). If a TTL level change is used for AZ, CELL OFF or STRT the voltage must be between 0 and 5V and the polarity sense of the device must match that of the detector (i.e., the "+" and the "-" of the external device must be connected to the "+" and "-" of the detector, respectively).

  The "+" terminal is the top one in each group and the "-" terminal is immediately below it, followed by ground.

⚠ CAUTION: When connecting TTL inputs, ensure that the potential across the terminals is set at 5 V DC (or less). When connecting outputs, ensure that the potential across the terminals is less than 30 V DC and the current is less than 0.5 A.

If desired, the baseline can be zeroed before injection of a sample by a momentary shorting of the AZ terminal posts. The signal should be sent by the autosampler or other external device.

If an autozero signal is used, activate it approximately 15 seconds before the injection to ensure that the autozero command is completed. When the autozero is triggered via the terminal block connection, an event marker can be provided after the output is zeroed.

⚠ CAUTION: The potential to the cells must be turned off if there is no fluid flowing through the cell. Failure to do so could damage the cell.

If desired, the operator can provide a signal to the detector from the pump (if the pump is so equipped) so that if the pump stops, an output signal is sent to the CELL OFF terminal which will turn the cells off (i.e., remove the potential) and prevent damage to the cells.

The ESA Model 582 Solvent Delivery Module has the capability of providing a contact closure in the event that it experiences an error. Types of errors registered by the pump include: the pump stops for some reason such as a mechanical or electrical problem, the back pressure of the HPLC system is outside the range of the upper or lower set pressure limits, the pump runs out of mobile phase, etc. This contact closure can be connected to the Coulochem III's CELL OFF input so that if an error is registered in the pump, the cells will be turned off to avoid damage.

To setup and activate this function in the Model 582 pump, it is necessary to connect the pump to the detector using one of the connection cables provided and set the external events function on the pump.

To connect the pump to the detector:

a) Insert the event cable plug into the pump EXT.CONTROL connector (upper left-hand side on the back panel).

b) Connect the orange and the yellow wires to the "+" and "-" terminals of the Coulochem III's CELL OFF I/O Connections.

c) Connect the black wire (common) to the GND terminal on the I/O Connections on the Coulochem III.

NOTE: It may be necessary to use the terminal block (supplied with the pump) and extra wires (not supplied) for the cabling to reach to the Coulochem detector, depending on how close your pump is to the detector.

To set external events on the pump:

a) While the pump is powered on, press the CE key to return to the initial screen.

b) Press FUNC key repeatedly until you come to the following screen:

0.0	EXT-S
Input	0 - 3

c) Input "2" and then press ENTER.

d) Press CE to return the initial screen.

This operation has set the Event 2 of the pump's Relay 2 (the Orange and Yellow wires) to present a contact closure in the event of a pump error. Now when a pump error occurs, the Coulochem III will turn the cells off.

If the pump turns the cells off, fix the pump (see Chapter 9, Troubleshooting, in the Model 582 Pump manual). After the pump has been fixed and is running normally, the cells can be turned back on.

NOTE: Please contact ESA [(800) 275-0102] or its representative with any questions concerning the connection of external devices to the Coulochem III.

- ***Power Cord Connection*** - The power cord (Part Numbers 70-1336 (100/120 V) or 70-1165 (220/240 V)) is inserted into the receptacle on the lower right of the rear panel.

- ***Thermal Organizer Module*** - If the Thermal Organizer Module (Part Number 70-5499TA) is included, the cable from the module is inserted into the connectors on the Power Supply Module and the Logic Module.

2.5.2 Connections for Testing the Detector Module

2.5.2.1 Detectors with a DC Potentiostat Module

To Prepare the Detector for Testing:

a) Connect the Simulator Test Load (Part Number 70-4755) to the DC potentiostat.

b) Plug the Guard Cell Simulator Test Load (Part Number 55-0172) into the Guard Cell Cable socket and the other end of the Guard Cell Cable to the Guard Cell connector on the rear of the detector.

c) Connect recording devices to the Signal Out 1 and Signal Out 2 ports on the DC potentiostat. Each channel should be connected to an output recording device. If this is not possible, it will be necessary to perform the test protocol for each channel on an individual basis. Ensure that the range of each recording device is 1 V full scale.

d) If the Coulochem III Thermal Organizer Module is included, plug the cable from it into the socket in the right-most panel on the above the power entry module and into the Logic Module.

e) Check the power input on the power entry module on the rear panel before connecting the detector to the mains. The Coulochem III Detector is configured for the voltage that meets the power requirements of the location to which it is shipped. Four voltage options are available at the power input (100/120/230 and 240 V). If it is necessary to change this setting, contact ESA Service.

f) Plug the power cord into the power module on the back of the detector and the mains.

g) Power up the instrument by depressing the power switch on the back of the unit.

h) Proceed to Section 2.6.

2.5.2.2 Detectors with a Pulse/Scan Module

To Prepare the Detector for Testing:

a) Connect the Simulator Test Load (Part Number 70-1790) to the Pulse/Scan Module.

b) Connect the recording device to the Signal Out connector of the Pulse/Scan Module. Set the range of the recording device to 1 V full scale.

c) Check the power input on the power entry module on the rear panel before connecting the detector to the mains. The Coulochem III Detector is configured for the voltage that meets the power requirements of the location to which it is shipped. Four voltage options are available at the power input (100/120/230 and 240 V). If it is necessary to change this setting, reference manual procedure.

d) If the Coulochem III Thermal Organizer Module is included, plug the cable from it into the socket in the right-most panel on the above the power entry module.

e) Plug the power cord into the power module on the back of the detector and the mains.

f) Power up the instrument by depressing the power switch on the back of the unit.

g) Proceed to Section 2.6.

2.6 Test Protocols

2.6.1 Establishing a Test Protocol

This section describes protocols that can be used to verify that the Coulochem III Detector and the potentiostats are functioning properly. These protocols are designed for initial system checkout and can also be used for troubleshooting purposes. Performing these protocols does not require a detailed understanding of the operating program of the detector.

☞ NOTE: If the Detector includes both the DC and the Pulse/Scan modules, both tests should be performed.

A detailed discussion of the operating program is described in Chapter 2 of the *Coulochem III (50W) User's Guide Manual*. Section 2.6.2 of this manual describes how to power up the detector and preliminary operations. The DC Mode Test Protocol (which is used for the DC module) is presented in Section 2.6.3 and the Scan Test Protocol (which is used for the Pulse/Scan module) is presented in Section 2.6.4.

☞ NOTE: ESA has developed a Validation Protocol which can be used to demonstrate that the Coulochem detector is operating in an acceptable manner. This protocol includes Installation, Operational and Performance Qualifications, and is performed by a factory trained service engineer. For details, please contact ESA or its Representative.

Worksheets to record the results of the checkout procedure are provided in Section 2.7.

If problems are observed, contact ESA or its representative. To assist the service engineer, please fax the worksheet to ESA's Service Department - Fax (978) 250-7092 - or your local representative to describe the problem and assist in the resolution of the problem.

2.6.2 Powering Up the Detector and Preliminary Operations

To Perform the Preliminary Operations:

a) Power up the Detector. The unit will undergo a warm-up period, followed by a series of internal diagnostic procedures. The DETECTOR WARMUP screen will be presented as shown in Figure 2-2. The program version number should be recorded for reference.

```
          COULOCHEM III SELFTEST
Testing xxxx Board
Program version xxxx
XXX Seconds remaining. Please wait.
```

Figure 2-2: The Detector Warm-up Screen

The system will check the DC module (if present), the Pulse/Scan module (if present) and calibrate the channels. During this time, the second line of the display will indicate the status of the testing procedure and the bottom line will indicate the period of time remaining for that test.

After the testing is completed, the copyright screen (Figure 2-3) will be presented.

```
  COULOCHEM III ELECTROCHEMICAL DETECTOR
Copyright 2000-2002  ESA Inc
All Rights Reserved.
                                  [NEXT]
```

Figure 2-3: The Copyright Screen

If a fault is determined during the diagnostic routine, a message will be presented on the display. If a fault message is presented, press the [RETEST] key. If the fault message is presented again, turn the power off, wait a few seconds and power the unit up again. If the message appears once again, contact the ESA Service Department or your local representative. Please provide the serial number of your detector, the software version level and the complete error message.

b) Press the NEXT key. The display will present the MAIN MENU screen (Figure 2-4) [the temperature will not be indicated if the Coulochem III Thermal Organizer Module is not connected to the detector module].

```
23°C     COULOCHEM MAIN MENU
EDIT:  Creates or edits a method.
RUN:  Runs a previously created method.
[SYSTEM]                [EDIT]    [RUN]
```

Figure 2-4: The Main Menu Screen

c) Press the [EDIT] key to present the EDIT METHOD SELECTION screen (Figure 2-5).

```
          EDIT METHOD SELECTION
Method Number: (1)  ""
Mode:Undefined       Date: -,-,- -:
[CANCEL]                         [EDIT]
```

Figure 2-5: The Edit Method Selection Screen

d) Press the [EDIT] key to present the MODE SELECTION screen (Figure 2-6).

```
              MODE SELECTION
This method's mode is DC
New Mode is(DC) with   (2)  Channel(s)
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 2-6: The Mode Selection Screen

2.6.3 The DC Mode Test Protocol

2.6.3.1 Establishing the DC Mode Test Protocol

To establish a DC Test Protocol:

a) Verify that *DC Mode* is indicated in the *Mode Selection* screen (Figure 2-6) and that the *Channels* field indicates the correct number of channels. If the *Mode* field does not indicate **DC**, press the ▲ or ▼ key until it does and then press ENTER. If the *Mode* field does not indicate two channels, press ENTER to access the field and use the ▲ or ▼ key to change the number to (2).

When the *Mode Selection* screen is correct, press [NEXT] to present the DC MODE screen (Figure 2-7).

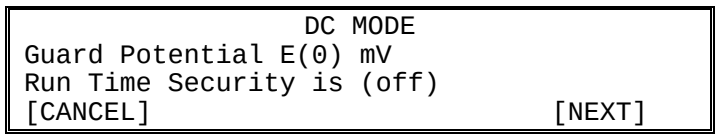

Figure 2-7: The DC Mode Screen

b) The cursor will appear in the *Guard Potential* field. Set the *Guard Potential* to 300 mV via the numerical keypad and press ENTER. The cursor will appear on the *Run Time Security* field. Verify that this field indicates off (it does not, press the ▲ or ▼ key to change it) and press [NEXT] to present the DC MODE CHANNEL 1 screen (Figure 2-8).

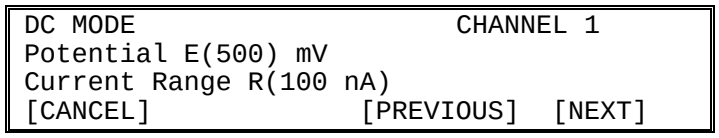

Figure 2-8: The DC Mode Channel 1 Screen - Page 1

c) Set the potential to 500 mV via the numeric keypad and press ENTER. The cursor will be placed on the *Current Range* field; use the ▲ or ▼ key to set the value to 100 nA and press ENTER, followed by the [NEXT] key to present the second page of the DC MODE CHANNEL 1 Screen (Figure 2-9).

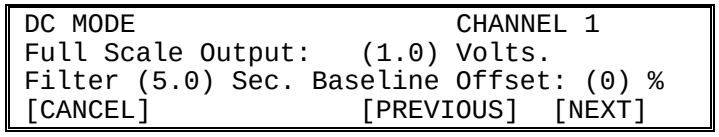

Figure 2-9: The DC Mode Channel 1 Screen - Page 2

d) Set the *Full Scale Output* to 1V using the ▲ or ▼ key and press ENTER, then set the *Filter* to 2 second using the ▲ or ▼ key and press ENTER, and finally set the *Baseline Offset* to 0 via the numeric keypad and press ENTER. Press [NEXT] to present the DC MODE CHANNEL 2 Screen - Page 1.

e) The parameters for channel 2 are the same as those for Channel 1, except that the potential should be set to -500 mV. After you have entered the appropriate parameters, press [NEXT] to present the SAVE METHOD screen (Figure 2-10).

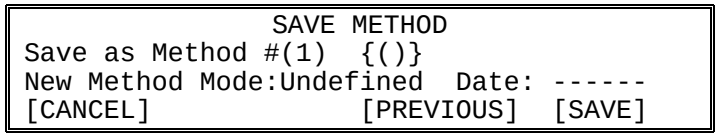

Figure 2-10: The Save Method Screen

f) Press [SAVE] to save the method. The method will be stored and the METHOD STORED screen (Figure 2-11) will be displayed.

```
                METHOD STORED
Saved as method 1
Select the NEXT key to continue
                                    [NEXT]
```

Figure 2-11: The Method Stored Screen

g) Press the [NEXT] key to return to the COULOCHEM MAIN MENU (Figure 2-4).

2.6.3.2 Running the DC Mode Test Protocol

To run the Test Protocol:

a) Access the COULOCHEM MAIN MENU (Figure 2-4) and press [RUN].

b) The SELECT METHOD screen (Figure 2-12) will be presented.

```
                SELECT METHOD
Method Number: (1)  ""
Type: DC     Date: March 1, 2002 12:34
[CANCEL]                          [RUN]
```

Figure 2-12: The Run Method Selection Screen

c) Press [RUN] to present the first DC METHOD RUNNING Screen (Figure 2-13). The CELLS ON/OFF led should illuminate indicating that you are applying the potentials to the external cells - in this case the Simulated Test Loads you installed as per paragraph 2.5.2.1.

```
             DC METHOD 1 RUNNING
E1(  500 mV) R(100nA) i  49.280nA   49%FS
E2( -500 mV) R(100nA) i -49.152nA  -49%FS
[STOP]   [EDIT]      [SETTINGS]   [GUARD]
```

Figure 2-13: The DC Method 1 Running Screen

Monitor the current for a short period of time. The currents should be approximately 50.0 ± 7 nA for channel 1 and -50 ± 7 nA for channel 2. Record the values on the DC Mode Worksheet (Section 2.7.1). The %FS values should be 50 ± 7%. Record these values on the DC Mode worksheet as well.

d) Press the [SETTINGS] key, which will present the SETTINGS screen (Figure 2-14).

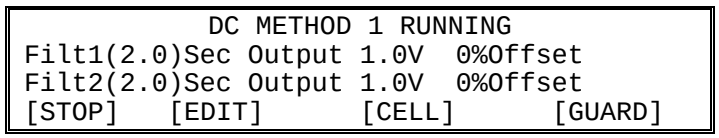

Figure 2-14: The DC Method 1 (SETTINGS) Running Screen

This screen displays the filter, full scale output voltage, and % baseline offset values you entered when you created Method #1.

e) Press the [GUARD] key to present the third DC METHOD 1 RUNNING Screen (Figure 2-15).

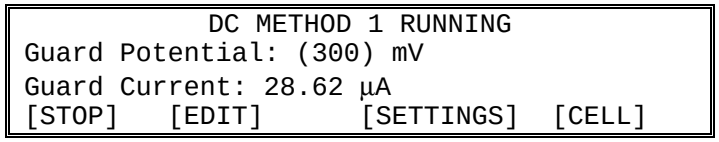

Figure 2-15: The DC Method 1 (GUARD) Running Screen

f) Monitor the Guard Current for a short period of time. The current should be 30 ± 3 µA. Enter the value on the DC Mode worksheet (Section 2.7.1).

g) Press the [CELL] key again to return to the Running Screen as shown in Figure 2-13. Press the AUTOZERO key once. You will see messages that Channel 1 and then Channel 2 are being autozeroed. In approximately 15 seconds, Figure 2-13 should be displayed again but now the ± 49% FS at the right end of the screen should say ± 0% FS. 0% FS should also be seen on the recording devices connected to the signal out 1 and signal out 2 BNC connectors on the rear panel.

If you are measuring the rear panel outputs with chart recorders the pens should now be resting on the zero line. Indicate that the recorder was reset on the DC Mode worksheet (Section 2.7.1).

h) Press the EVENT MARK key. An instantaneous signal of +5% for both channels should be placed on the trace (if the direction is reversed, then the cable leads to the two channels of the recording device are probably reversed). Indicate that the event mark was observed on the DC Mode worksheet (Section 2.7.1).

If you have performed the test only on Channel 1 (i.e., only one recording device is available), repeat the test for Channel 2.

i) Press the CELLS ON/OFF key on the keypad. The illuminated LED should go out. Monitor the Guard Cell current. The current should be 300 ± 15 µA.

NOTE: Wait about 10 seconds for the signal to stabilize before recording the current reading.

j) If the detector is fitted with a Heated Organizer, press SYSTEM to present the SYSTEM menu and then press the ▲ or ▼ key to access the THERMAL ORGANIZER SETUP Screen (Figure 2-16).

```
          THERMAL ORGANIZER SETUP
Temperature Control (Off)
Thermal Organizer is Off
[CANCEL]   [RESTORE]    [SAVE]
```

Figure 2-16: The Thermal Organizer Setup Screen

k) Change the *Temperature Control* field to *On* with the ▲ or ▼ key. This will present a field to enter the desired temperature. Use the numerical keypad to enter a temperature 10°C above ambient, press ENTER and then press [SAVE].

l) The system will store the new temperature and will provide power to the heater to raise the temperature. While the temperature is increasing, the present temperature will be indicated with an up arrow (e.g., 33↑C). Indicate on the worksheet that the temperature rose to the desired level and stabilized.

2.6.4 The Pulse/Scan Mode Test Protocol

2.6.4.1 Establishing the Pulse Mode Test Protocol

To establish a Pulse Test Protocol:

a) Place the Simulator Test Load (Part Number 70-1790) on the cell connector of the Pulse/Scan Module.

b) Verify that Pulse Mode is indicated in the Mode Selection screen (Figure 2-6). If the Mode field does not indicate Pulse, press the ▲ or ▼ key until it does and then press ENTER. When the Mode Selection screen is correct, press [NEXT] to present the PULSE EDIT Screen #1 (Figure 2-17).

```
                PULSE EDIT
Filter(None)
Run time security is (off)
[CANCEL]                        [NEXT]
```

Figure 2-17: The Pulse Edit Screen #1

c) Select the None option for Filter and ensure that Run time security is off then press [NEXT] to present the PULSE EDIT Screen #2 (Figure 2-18).

```
                PULSE EDIT
Potential 1 {1000}mV Time 1 {800} mS
Potential 2 {  0}mV        Time 2 {100} mS
[CANCEL]           [PREVIOUS]   [NEXT]
```

Figure 2-18: The Pulse Edit Screen #2

d) Set Potential 1 to 1000 mV via the numerical keypad and press ENTER. The cursor will appear on the Time 1 field. Set the Time to 800 msec via the numerical keypad and press ENTER. Repeat the process for the Potential 2/Time 2 fields, which should be set to 0 mV for 100 msec. After you have entered the parameters, press [NEXT] to access the PULSE EDIT Screen #3 (Figure 2-19).

```
                PULSE EDIT
Potential 3 {0}mV    Time 3 {100} mS
Potential 4 {0}mV    Time 4 {0} mS
[CANCEL]           [PREVIOUS]   [NEXT]
```

Figure 2-19: The Pulse Edit Screen #3

e) Enter 0 mV for Potentials 3 and 4, and 100 msec for Times 3 and 4. Then press [NEXT] to present the PULSE EDIT Screen #4 (Figure 2-20).

```
                PULSE EDIT
Acq. Delay (300 mS)  Offset(     0) %
Current Range R(100nC) FS Output  (1.0) V
[CANCEL]           [PREVIOUS]    [NEXT]
```

Figure 2-20: The Pulse Edit Screen #4

f) Enter a 300 msec Acquisition Delay using the numeric keyboard and press ENTER. Set the Offset to 0 and press ENTER. Set the Range to 100 nC via the ▲ or ▼ key and press ENTER and finally set the FS output to 1.0 V via the ▲ or ▼ key. After all of the parameters have been set, press [NEXT] to present the SAVE METHOD Screen (Figure 2-21).

```
                SAVE METHOD
Save as Method #(1)        {()}
New Method Mode:Undefined     Date: ------
 [CANCEL]          [PREVIOUS]      [SAVE]
```

Figure 2-21: The Save Method Screen

g) Press [SAVE] to save the method. The method will be stored and the METHOD STORED screen (Figure 2-22) will be displayed.

```
                METHOD STORED
Saved as method 1.
Select the NEXT key to continue
                                    [NEXT]
```

Figure 2-22: The Method Stored Screen

h) Press the [NEXT] key to return to the COULOCHEM MAIN MENU (Figure 2-4).

2.6.4.2 Running the Pulse Mode Test Protocol

To run the Test Protocol:

a) Access the COULOCHEM MAIN MENU (Figure 2-4) and press [RUN].

b) The SELECT METHOD screen (Figure 2-23) will be presented.

```
                SELECT METHOD
Method Number (1)    ""
Type: Pulse      Date: March 1, 2002 12:34
[CANCEL]                          [RUN]
```

Figure 2-23: The Select Method Screen

c) Press [RUN] to present the PULSE METHOD 1 RUNNING Screen (Figure 2-24).

```
        PULSE METHOD  1  RUNNING
E{1000} mV    T1(800}ms: AD{20}
R(100 nC) 50.00 nC 50%FS F{None} O( 0)%
[STOP]       [EDIT]     [PULSE 2-4]
```

Figure 2-24: The Pulse Method 1 Running Screen

The charge, Q (the value immediately after the range), should be 50.00 ± 5.00 nC and % FS should be 50 ± 5. The observed values should be entered in the Pulse Mode Worksheet (Section 2.7.2).

d) Press the AUTOZERO key once to present a message that the system is being autozeroed. In about 5 seconds, Figure 2-23 is displayed again but now the ± xx%FS at the right end of the screen should say ± 0% FS. The value 0% FS is also be seen on the recording devices connected to the Signal Out connector on the rear panel. If you are measuring the rear panel outputs with chart recorders, the pens should now be resting on the zero line. (Previously it was 50% of scale.) Indicate that the recorder was reset (Pulse Mode Worksheet (Section 2.7.2).

e) Press the EVENT MARK key. An instantaneous signal of +5% should be placed on the trace (if the direction is reversed, then the cable leads to the recording device are probably reversed). Indicate that the event mark was observed on the Pulse Mode Worksheet (Section 2.7.2).

f) Press the CELLS ON/OFF key on the keypad. The illuminated LED should go out and the charge should be in the overrange (#######) condition.

g) If the detector is fitted with a Thermal Organizer, press SYSTEM to present the SYSTEM SETUP MENU and then press the ▲ or ▼ key to select the THERMAL ORGANIZER screen.

h) Press the [NEXT] key to access the THERMAL ORGANIZER SETUP Screen (Figure 2-25).

```
        TEMPERATURE ORGANIZER SETUP
Temperature Control (Off)
Thermal Organizer is Off
[CANCEL]              [RESTORE]    [SAVE]
```

Figure 2-25: The Thermal Organizer Setup Screen

i) Change the *Temperature Control* field to *On* with the ▲ or ▼ key. This will present a field to enter the desired temperature. Use the numerical keypad to enter a temperature 10°C above ambient, press ENTER and then press [SAVE].

j) The system will store the new temperature and will provide power to the heater to raise the temperature. While the temperature is increasing, the present temperature will be indicated with an up arrow (e.g., 33↑C). Indicate on the worksheet that the temperature rose to the desired level and stabilized.

2.7 ESA Coulochem III Worksheets

This section includes the worksheets for the DC Mode (Section 2.7.1) and the checkout form for the Pulse Mode (Section 2.7.2). The user may copy these forms as desired (i.e., as a protocol to check out the detector as part of a troubleshooting procedure).

2.7.1 DC Mode

ESA COULOCHEM III WORKSHEET - DC MODE
In Case of Problems, Please Contact the ESA Service Department or Your Authorized ESA Dealer

GENERAL INFORMATION

Organization _____ Operator _____
Address _____ Phone Number _____
_____ Fax Number _____
_____ Email Address _____
Date _____

Detector Unit S/N _____ Potentiostat S/N _____
Logic Module S/N _____ Software Version _____

A) General Information

If there is an error message on the display when the unit is powered up, please indicate.

_____

Do the LEDs illuminate when appropriate?

Scroll (between ▲▼) _____ Security _____
Cells ON/OFF _____ Remote _____

B) DC Mode Tests

Current Tests

	Present Value	**Expected Value**
Guard Cell Current (Cells Out)	_____	300 ± 15 µA

NOTE: ***Make sure the Cells In/Out activity indicator light is lit before recording values below.***

	Present Value	**Expected Value**
Guard Cell Current	_____	27 to 33 µA
Cell 1 Current	_____	43 to 57 nA
Cell 2 Current	_____	-43 to -57 nA
Cell 1 % FS	_____	43 to 57%
Cell 2 % FS	_____	-43 to -57 %

Autozero/Event Mark Tests (Yes/No)

	Recorder Pen Autozero?	Recorder Pen Event Mark?
Cell 1	_____	_____
Cell 2	_____	_____

Thermal Organizer Test (Yes/No)

Thermal Organizer temperature increased and stabilized at set temperature: _____ °C

Remarks _____
_____

2.7.2 Pulse Mode

ESA COULOCHEM III WORKSHEET - PULSE MODE
In Case of Problems, Please Contact the ESA Service Department or Your Authorized ESA Dealer

GENERAL INFORMATION

Organization _____ Operator _____
Address _____ Phone Number _____
_____ Fax Number _____
_____ Email Address _____
Date _____

Detector Unit S/N _____ Potentiostat S/N _____
Logic Module S/N _____ Software Version _____

A) General Information

If there is an error message on the display when the unit is powered up, please indicate.
_____

Do the LEDs illuminate when appropriate?:

Scroll (between ▲▼) _____ Security _____
Cells ON/OFF _____ Remote _____

B) Pulse Mode Tests

NOTE: ***Make sure the Cells In/Out activity indicator light is lit before recording values below.***

	Present Value	**Expected Value**
Q (charge)	_____	45 to 55 nC
% FS	_____	45 to 55 %
	Autozero Value	
Q (charge)	_____	45 to 55 nC
% FS	_____	-1 to 1 %

Autozero/Event Mark Tests (Yes/No)

	Initial Pen Position (%)?	Recorder Pen Autozero?	Recorder Pen Event Mark?
Action	_____	_____	_____

Thermal Organizer Test (Yes/No)

Thermal Organizer temperature increased and stabilized at set temperature: _____ °C

Remarks _____
_____

3 Installing the Detector in a HPLC System

3.1 Introduction

This chapter describes how the detector is installed into a high performance liquid chromatographic system and includes:

- A discussion of various activities that may be required to install your system such as passivation, cutting tubing, and how a fitting is made. (Section 3.2).
- Step by step installation of each component in the system (Section 3.3).
- Equilibrating the system and preparing it for analytical work (Section 3.4).

A functional diagram of a typical HPLC system with a Coulochem® III Electrochemical detector is shown in Figure 3-1. Some of the components (e.g., the Guard Cell and the conditioning cell) are optional and are used for specific applications.

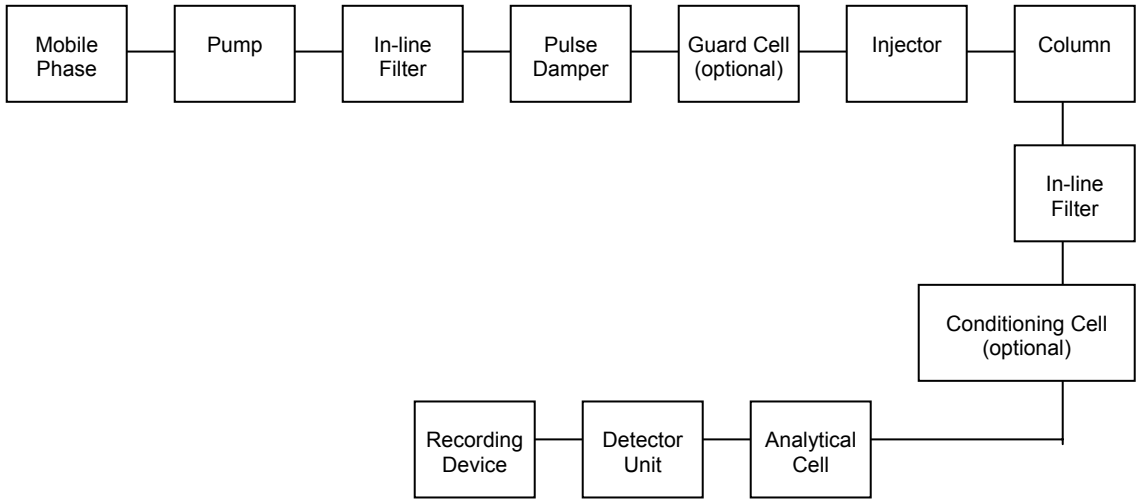

Figure 3-1: Typical Fluidics Configuration

If other modes of detection (e.g., absorbance, fluorescence) are used in conjunction with an electrochemical cell, the flow cell(s) for these detectors are usually placed after the electrochemical cell (unless an electrochemical reaction changes the compound of interest).

There are two reasons for this configuration:

- Some detectors have a larger flow cell than the electrochemical cells provided by ESA. This will create greater band spreading than the flow cell(s) provided for the electrochemical detector.

- Detector cells restrict the flow of the mobile phase and create backpressure. The Coulochem III analytical cell is designed to withstand pressures of up to 41 bar (600 psi). If the electrochemical cell is placed after a cell with a lower pressure limit, it is possible that the upstream flow cell could be damaged.

☞ NOTE: If an electrochemical cell is placed after another cell, check with the manufacturer of the other cell to ensure that the cell can withstand the elevated pressure (typically 6-15 bar).

3.1.1 General Approach to Installing the Detector into an HPLC System

When a Coulochem III detector is incorporated into an HPLC, the strategy is to start from the solvent delivery system, add a component and then check for leaks. This approach has been found to be very useful in isolating problems and minimizing the overall effort for installation of the system. Each component of the system (e.g., solvent delivery module, autosampler) should be checked on a local basis as described in the manual supplied with the unit before installing it in the system (a check-out protocol for the Coulochem III Electrochemical Detector is presented in Chapter 2).

☞ NOTE: A mixture of degassed methanol/water (20/80) can be used as the mobile phase for the installation protocol instead of the mobile phase to be used for the actual analysis. This mobile phase should be filtered through a 0.22 µm filter and degassed.

To minimize chromatographic band broadening, it is critical that narrow bore PEEK™ tubing (0.005" ID [which is red], Part Number 70-0491) or (0.007" ID tubing [which is yellow], Part Number 70-0492) is used downstream from the injector. In addition, the length of the tubing is to be minimized. The analytical cells should be placed as close as possible to the end of the analytical column to minimize post column band broadening.

As you install the system, it is critical to maintain a clean working environment and ensure that particulate matter does not enter the system. The presence of particulate matter will reduce the effectiveness of the cells and may lead to premature replacement of various components.

⚠ WARNING: If the unit is used in a manner not specified by ESA, the protection provided by the equipment may be impaired.

3.2 Preliminary Activities

3.2.1 Passivation

Some liquid chromatographic systems include a significant number of components that are made from polished stainless steel (pump components, tubing, fittings, pulse dampers, frits, etc.). The surfaces of these components include oxides of iron that may slowly be dissolved or otherwise removed by the mobile phase. Since these oxides may be electrochemically active and may create high background currents and/or drifting baselines, it may be necessary to thoroughly clean (passivate) certain components of the chromatograph to maximize the performance of the detector.

☞ NOTE: ESA Solvent Delivery Modules and peripherals supplied by ESA are manufactured from components that do not require passivation.

If components of the HPLC system are fabricated from metals other than Type 316 Stainless Steel, it is likely that it will be necessary to passivate the system before use. The user should refer to the specifications documentation for each component to be placed in the system or contact the manufacturer for information.

⚠ CAUTION: Before attempting to passivate the instrument, check with the manufacturer of the HPLC pump and/or injector to ensure that the procedure described below will not adversely affect any component.

⚠ CAUTION: Failure to passivate systems that contain metals other than Type 316 stainless steel may irreversibly damage coulometric cells and will void the warranty for ESA flow cells.

If it is necessary to passivate a component:

a) Pump HPLC grade water through the component at a flow rate of 1 mL/min for approximately 30 minutes.

⚠ CAUTION: If you are passivating a solvent delivery module or any other component that is incorporated into an existing system, remove the pre-column, the column and any electrochemical cells before starting the passivation process.

b) If the chromatographic system contains residual levels of organic materials that are not water soluble, flush the system with an organic solvent such as methanol to remove them. After flushing the system with the organic solvent, pump HPLC grade water through the system as described above before starting the passivation process. If the mobile phase that is presently in the system is not miscible with water, gradually change its composition so that it will become miscible with water.

c) Pump 6N Nitric Acid through the system at a flow rate of 1.0 mL/min for approximately 30 minutes.

An alternative passivation procedure involves the use of a 0.05M EDTA (ethylenediaminetetraacetic acid) solution, pH = 8.0, instead of the nitric acid in step (c).

⚠ WARNING: Nitric acid can cause personal injury. Wear protective clothing and use eye protection.

d) Pump HPLC grade water through the system at a flow rate of 1.0 mL/min until the pH of the eluent is approximately 5.0.

☞ NOTE: Small components such as ferrules, metal tubing and fittings can be passivated in the manner described above using a syringe to flush the components.

3.2.2 Cutting Tubing and Making Fittings

Stainless steel or PEEK tubing can be used to connect the various components of the system. If stainless steel tubing is used, it may need to be passivated as described above. PEEK tubing is provided for the various connections in the system and is included with some components (e.g., the cell kit contains pre-cut PEEK tubing to connect the cell to the next cell in the series).

☞ NOTE: Stainless steel tubing supplied by ESA has been passivated and it is not necessary to be passivated by the user.

To cut PEEK tubing:

a) Insert the PEEK tubing in the appropriate guide hole in the tubing cutter (Part Number 70-1307) and position the tubing to the desired length.

b) Release the tabs on the cutter.

c) Spin the cutter a few times; remove the tubing and snap the desired piece off. As an alternative, you can continue to rotate the cutter around the tubing to allow the cutter to finish the cut.

d) Inspect the tubing to ensure that the inner channel of both ends is round. (If the inner channel is not properly cut, it is likely that the flow through the tube will be restricted.)

e) Connect tubing to the system as described below and flush with mobile phase or solvent.

NOTE: Use PEEK tubing with an internal diameter if 0.010" or less after the injector. In addition, minimize the tubing length after the injector; failure to do so may result in poor chromatographic resolution.

To make a fitting:

a) Turn off the flow of mobile phase before making a fitting (if the tube is connected to the system).

b) Insert the tubing through the fitting and push the tubing so that is firmly seated in the receptacle (Figure 3-2). (This figure shows a "flat bottom" ferrule type. A "cone shaped" ferrule fitting is made in a similar manner.)

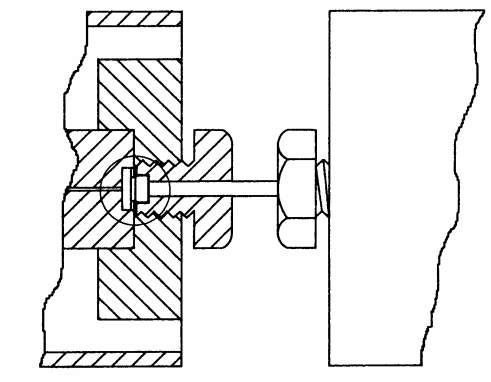

Figure 3-2: Seating the Tube Assembly

c) Finger tighten the fitting and initiate flow through the system.

⚠ CAUTION: Do not overtighten the fitting, as that may lead to permanent deformation of the ferrule.

d) Monitor the joint. If a leak is observed, tighten the fitting to stop any leaks. To ensure that a good fit is made, gently tug on the tubing after the connection has been made.

3.3 Configuring the HPLC System with an Electrochemical Detector

3.3.1 Installing the Solvent Delivery System

The solvent delivery system refers to the components that are upstream of the sample injector (Figure 3-3).

Figure 3-3: Solvent Delivery System

The solvent delivery system includes the following items:

- **Solvent delivery module (pump)**.
- **In-line filter** which is used to ensure that particulate matter from the solvent delivery module does not enter the system.
- **Pulse damper** which contains a reservoir of methanol or isopropanol and a chemically resistant membrane.
- **Guard cell** which is used to oxidize (reduce) electroactive compounds in the mobile phase *(Optional)*.

The filter, pulse damper and guard cell can be installed in the ESA Coulochem III Organizer Module (optional) or ESA Coulochem III Thermal Organizer Module (optional). This module allows the user to securely mount up to three cells, a pulse damper, a column and an injector.

Technical data describing the ESA Coulochem III Organizer Module and ESA Coulochem III Thermal Organizer Module and how they are used to mount various configurations is presented in Chapter 14. After each component has been attached to the system, re-start the delivery of the degassed solution of mobile phase (e.g., MD-TM) or Methanol/Water (20/80). Allow it to flow through the system for 3-5 minutes at a flow rate of 1.0mL/min to expel the air in the system. Monitor the fittings and tighten as appropriate. If a significant increase in pressure is observed, check to determine if an obstruction or clogged component is present.

The flow rate should be increased in a series of small increments while monitoring the pressure. If it appears that the pressure is rising rapidly, discontinue the flow and check for clogged or blocked tubing and/or connections. Once the system appears to be functioning properly and no leaks are observed, turn the solvent delivery module off and proceed to the next step.

To install the Solvent Delivery System:

a) Turn on the solvent delivery module at the proper flow rate (see Table 3-1).

Table 3-1: Flow Rate for Various Columns

Column Diameter	Typical Flow Rate
4.6 mm	1.0 mL/min
3.0 mm	0.5 mL/min
2.0 mm	0.2 mL/min
1.0 mm	0.075 mL/min

b) Connect the outlet line from the solvent delivery module (or mixer, if appropriate) to the inlet of the in-line filter using the tubing supplied with the filter. In addition, attach tubing to the down-stream side of the filter holder. The filter holder should be installed with direction of the arrow pointing downstream. Open the filter holder and insert a graphite filter. The pre-filter kit includes a holder and 5 graphite filters (Part Number 70-0898). The in-line filter is used to ensure that particulate matter from the pump does not does not enter the system. Connect tubing from the solvent delivery module to the graphite filter holder.

c) If a pulse damper (Part Number 70-0894) is included in the system, connect the downstream tubing from the in-line filter to the pulse damper and attach tubing to the down stream end of the pulse damper.

d) If a Guard Cell (Part Number 55-0417) is included in the system, connect the downstream tubing from the in-line filter to the inlet side of the Guard Cell. After the Guard Cell is attached to the system, flush it with approximately 5 mL of mobile phase then turn the flow of mobile phase off.

3.3.2 Installing the Injector, Pre-Column, Analytical Column and Post Column In-line Filter

To install the Injector, Pre-Column, Analytical Column, and Post Column In-line Filter:

a) Connect the downstream tubing from the in-line filter or Guard Cell (if installed) to the inlet of the manual (or automated) injector and connect tubing to the outlet from the injector. Re-start the delivery of the mobile phase. Allow the degassed methanol/water solution to flow for 3-5 min at a flow rate of 1.0 mL/min to expel air from. Monitor the fittings and tighten as appropriate. If a significant increase in pressure is observed, check if an obstruction or clogged component is present.

b) If a pre-column is employed, connect it to the downstream tubing from the injector (observe the indicated direction of flow for the pre-column) and attach tubing to the down stream end of the pre-column. Re-start the delivery of the methanol/water mixture. Allow the mobile phase to flow through the system without the column for 3-5 minutes at a flow rate of 1.0 mL/min to expel the air in the system. Monitor the fittings and tighten as appropriate.

☞ NOTE: Use PEEK filter elements (Part Number 70-3824) in the in-line prefilter holders located after the injector. Graphite filter elements (Part Number 70-0898) should be used before the injector.

☞ NOTE: When the pre-column is installed, an increase in back pressure will be observed. Check each fitting in the system for leaks and tighten the fittings (if necessary).

c) Connect the analytical column to the downstream tubing from the injector or pre-column and attach waste tubing to the down stream end of the column. Re-start the delivery of the mobile phase (the flow rate is dependent on the internal diameter of the column as indicated in Table 3-1).

☞ NOTE: Make certain that the column is installed so that the flow direction is correct. Typically an arrow on the column indicates the correct flow direction.

Flush the column to waste for 10-15 min before connecting the next component in the system. Monitor the fittings and tighten as appropriate.

NOTE: A detailed discussion of the installation of a new column is presented in Technical Note 70-1784 - included as Appendix C.

NOTE: When the analytical column is installed, an increase in back pressure will be observed. Check each fitting in the system for leaks and tighten the fittings (if necessary).

d) Replace the methanol/water solution with mobile phase and pump this through the column to waste for several hours or overnight before attaching the analytical cell.

e) Connect an in-line filter after the analytical column (see Section 3.3.2a) for details). A PEEK filter (Part Number 70-3824) should be used in this filter holder instead of the graphite filter used before the injector. Re-start the delivery of the mobile phase and allow the mobile phase to flow through the system for 3-5 minutes to expel the air in the system. Monitor the fittings and tighten as appropriate.

3.3.3 Attaching Electrochemical Cells to the System

To attach electrochemical cells to the system:

a) If the system includes a Model 5021 Conditioning Cell, connect the downstream tubing from the post-column in-line filter. Re-start the delivery of the mobile phase and allow the mobile phase to flow through the system for 3-5 minutes at the appropriate flow rate (see Table 3-1) to expel the air in the system. Monitor the fittings and tighten as appropriate.

b) Connect the Analytical cell to the downstream tubing from the post-column in-line filter or the Conditioning Cell. Make certain that the IN and OUT connections are correctly made, and the flow is in the direction indicated on the cell. Re-start the delivery of the mobile phase and allow the mobile phase to flow through the system for 3-5 minutes at the appropriate flow rate (see Table 3-1) to expel the air in the system. Monitor the fittings and tighten as appropriate.

c) If the system includes two (or more) cells, add the cells in the same manner as described in step (b).

d) If a Model 5014B or Model 5041 cell in included in the system, purge the reference port by allowing mobile phase to flow through it and out the purge port at the appropriate flow rate (see Table 3-1) for a few minutes before allowing the cell flow to go through the cell outlet tubing. After the cell has been purged, close the purge port with the dead-end fitting.

3.4 Preparing the System for Operation

3.4.1 Equilibrating the System

After the system is operating at the desired flow rate, pump the mobile phase through the system for at least a half hour, monitoring the pressure and check for leaks. The high pressure cut off control on the pump should be set at approximately 35 bar (500 psi) *above* the system pressure observed at this time. If the Coulochem III Thermal Organizer Module is included, allow sufficient time for thermal equilibration.

If a Model 5040 or 5041 Analytical Cell is used, the working electrode should not need to be polished before being used for the first time. However, if polishing is desired instructions are included in the polishing kit for the cell and are included in Section 6.4.2.

3.4.2 Preparing the Analytical Column

The column to be used for the analysis can be prepared for use with an electrochemical detector by either of the following procedures. A detailed discussion of HPLC column installation is presented in Appendix C.

☞ NOTE: The aqueous portion of the mobile phase used in this procedure should be filtered through a 0.22 µm membrane (Nylon or PVDF) filter, mixed with the organic component and degassed before use.

3.4.3 Equilibrating the Cells

To equilibrate the cells:

a) Connect the various cells to the detector unit via the appropriate cable and power up the detector unit.

b) Access DC mode and set the potential to the value(s) required for the application. Monitor the current; initially the current will be fairly high and there will probably be considerable noise. As the cell equilibrates, background currents and noise will decrease. The period of time required for equilibration will depend on the desired gain/sensitivity and can be several hours.

4 Theory of Operation

4.1 Basics of Electrochemistry

Electrochemistry involves chemical reactions in which an electron is transferred from one compound (element or ion) to another. Typical electrochemical reactions are shown in Equations 4-1 and 4-2.

$\mathbf{HQ \rightarrow Q + 2e^- + 2H^+}$	**(oxidation)**	**4-1**
$\mathbf{Q + 2e^- + 2H^+ \rightarrow HQ}$	**(reduction)**	**4-2**
Hydroquinone = HQ	**Quinone = Q**	

Equation 4-1 describes the oxidation of hydroquinone (HQ), while Equation 4-2 describes the reduction of quinone (Q). In an oxidation, the compound loses an electron(s); while in a reduction, the compound gains an electron(s). In order for an oxidation or a reduction to occur, energy in the form of an electric potential is required. The processes described in Equations 4-1 and 2 are termed *half reactions* and a pair of species involved in an electrochemical reaction is called a *redox couple* (i.e., HQ and Q in this example). When electrochemical detection is used, the analyst typically is concerned with only one process (e.g., the oxidation of HQ).

When an electrochemical process is desired, a pair of inert electrodes are placed in a cell and a potential is placed across the electrodes (Figure 4-1). If the potential is great enough, the reaction will occur and current will flow. The magnitude of this current is related to the concentration of the compound in solution.

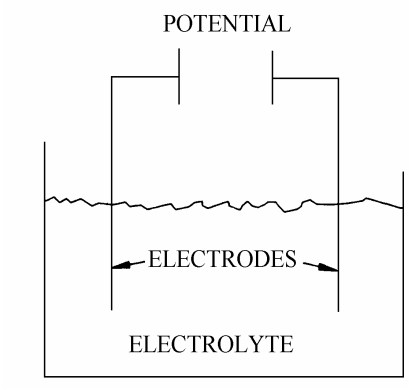

Figure 4-1: Configuration of an Electrochemical Cell

In DC mode, the current is measured as the potential across the cell is held constant (DC mode). The electrode where the desired electrochemical process occurs is termed the working electrode, while the electrode where the complementary electrolytic reaction takes place is called the auxiliary or counter electrode. A reference electrode is included to provide a stable potential between the working electrode and the counter electrode.

In this chapter, we will describe the fundamentals of electrochemical detection and explain how the DC modes of electrochemical detection can be used to monitor the eluant from an HPLC column. A variety of other modes are available including Pulse Mode (Chapter 12), Screen Mode (Chapter 10), Scan Mode (Chapter 13), and Redox Mode (Chapter 11).

4.2 What Potential is Needed for an Electrochemical Reaction

4.2.1 Current/Voltage Curves or Hydrodynamic Voltammograms

The successful use of an electrochemical detector for liquid chromatography (LCEC) requires knowledge of the potential to effect the desired electrochemical reaction (i.e., oxidation or reduction of the species of interest). This potential is dependent on a large number of factors including the nature of the electrode surface, the pH, the composition of the mobile phase and the chemical structure of the species of interest.

The appropriate potential for electrochemical detection can be determined by measuring the oxidative (or reductive) current of the analyte (at a constant concentration) over a range of working electrode potentials. A plot of current generated (peak height) vs. applied potential is commonly called a hydrodynamic voltammogram (HDV) or a current/voltage (C/V) curve. A typical HDV is shown in Figure 4-2.

Visual inspection of the curve indicates the potential where the oxidative (or reductive) current is largest for both the oxidative and the reductive modes. Since the concentration of the sample is fixed in this experiment, the current/voltage curve can be used to find local maxima.

In Figure 4-2, it can be seen that the cathodic current (corresponding to reduction) appears to plateau at 100 mV.

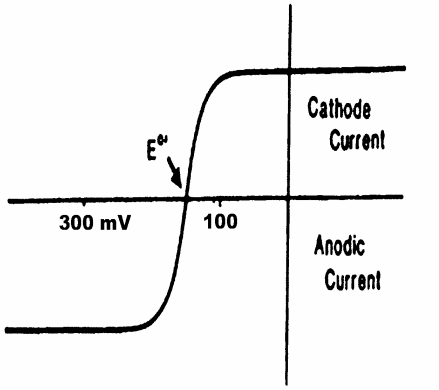

Figure 4-2: A Current/Voltage (C/V) Curve for a Redox Couple

As an alternative, tables of standard electrode potentials can be used to determine a suitable starting point for the potential of a given reaction. The standard electrode potential for a given half reaction can be determined by using the half reaction potentials of a cell containing the appropriate redox couple (e.g., HQ/Q) and the standard hydrogen electrode. Any standard analytical chemistry text can be consulted for a discussion about the use of standard electrode potentials. If the potential for a process has been determined using the tables of standard electrode potentials, a current/voltage curve should be obtained to find the optimum potential to maximize the sensitivity.

NOTE: A current/voltage curve should be obtained for the compound(s) of interest when a system is initially setup and a new curve should be obtained when a component of the system (e.g., the cell) is replaced or if the chromatography is significantly changed.

4.2.2 The Nernst Equation

The Nernst equation (Equation 4-3) is the primary relationship which describes the potential of an electrode immersed in an electroactive solution as a function of the concentration of the electroactive species present.

$$E_{app} = E° - \frac{0.059}{n} \log \frac{[ox]}{[red]} \qquad 4\text{-}3$$

where: E_{app} is the applied potential.

E° is the standard reduction potential for the oxidation/reduction couple (25°C, 1 atm).

[ox] is the concentration of the oxidized form of the compound(s) in the reaction at the surface of the electrode.

[red] is the concentration of the reduced form of the compound(s) of interest at the surface of the electrode.

n is the number of electrons that are transferred in the electrochemical process.

The concentration of a species that is not in solution but in intimate contact with the solution (e.g., a gas or a solid) is considered to be unity (1).

The appropriate form of the Nernst equation for a typical reaction (e.g., the oxidation of hydroquinone (HQ) described in Equation 4-1) is shown in Equation 4-4:

$$E_{app} = E° - (0.059/2) \log ([HQ]/[Q][H^+]^2) \qquad 4\text{-}4$$

The Nernst equation shows that the potential for this process is pH dependent. In a well-buffered solution, the pH will remain constant and the relationship between potential and the concentration of the oxidized and reduced form is shown in Equation 4-5.

$$(2/0.059)\,(E°' - E_{app}) = \log ([HQ]/[Q]) \qquad 4\text{-}5$$

where: **E°'** is E° - 0.059 (pH)

4.3 The Role of Diffusion in Electrochemical Processes

The Nernst equation indicates that if the applied potential (E_{app}) is greater than E°, the surface concentration of HQ must be small compared to the surface concentration of Q in order for the reaction to proceed as written. When HQ passes through the cell, it will be oxidized and the observed current will rise as the applied potential is increased. This increase will depend on the electrochemical characteristics of the compound of interest and the nature of the electrode material.

An important point in the detection is that as HQ is oxidized to Q, fresh HQ must be brought to the surface in order for the reaction to proceed and Q must be removed from the electrode surface. This phenomenon can be best understood by dividing the overall oxidation process into three discrete steps, as shown in Equations 4-6 to 4-8.

1) Mass Transport	**HQ (solution) → HQ (surface)**	**4-6**
2) Electron Transfer	**HQ (surface) → Q (surface) + $2H^+$ + $2e^-$**	**4-7**
3) Mass Transfer	**Q (surface) -→ Q (solution)**	**4-8**

Equation 4-6 describes the diffusion of HQ from the bulk solution to the surface of the electrode, while Equation 4-8 describes the convection of Q from the surface of electrode to the bulk solution. Since diffusion and convection are relatively slow compared to the actual oxidation of HQ shown in Equation 4-7, they can be the limiting processes in an electrochemical process and influence the effectiveness of electrochemical detection for HPLC.

At some voltage, an increase in the applied potential will *no longer* lead to an increase in the observed current. This current is termed the limiting current and is proportional to concentration of HQ in the sample as well as the size and composition of the electrode. The limiting current is a function of the rate of the processes described in Equations 4-6 to 4-8, which are diffusion related.

Many of the electrodes for the ESA Coulochem® III detector are made from very porous graphite and have an extremely large surface area. Since the eluent flows <u>*through*</u> the electrodes (rather than *by* the electrode), the contact of the electroactive compound in solution with the electrode surface is maximized. The fact that the eluent flows *through* large surface area electrodes (rather than *by* electrodes with a much smaller surface area) significantly increases the rate of diffusion of the electroactive compound. Similarly, the convection of the reaction products away from the electroactive surface of the electrode is a significant factor in maximizing the electrochemical response.

Again, the fact that the mobile phase flows through electrodes with a large surface area rather than by an electrode with a smaller surface area significantly increases the rate of convection of the reaction products from the electrode.

To summarize, the cell design, the large surface area of the electrodes and the fact that the eluent actually flows through porous graphite electrodes ensures that diffusion and convection controlled processes do not limit the electrochemical response of the detector. This allows complete oxidation (reduction) of the electroactive compound in solution and maximizes the sensitivity of the analysis. Since this type of cell provides essentially complete conversion of the electroactive species, a detector which incorporates porous graphite electrodes is a coulometric detector (rather than as an amperometric detector).

NOTE: Extremely high concentrations of analyte (e.g., > 100 µg/mL) or extremely fast flow rates can overwhelm an electrode, thereby allowing some unreacted species to pass through the electrode.

4.4 The Reversibility of Electrochemical Reactions

Many electrochemical reactions are reversible. A reversible redox couple meets the following chemical and thermodynamic conditions:

- A reversible couple is present when the oxidized and reduced forms of a compound are in chemical equilibria with each other. The hydroquinone-quinone couple and the ferricyanide-ferrocyanide couple are examples.

- A small change in the potential may change the direction of the reaction. This occurs near $E^{\circ\prime}$ (but not at potentials which produce the limiting current).

When an electrochemical reaction starts, only one species is present. As soon as some of the compound of interest has been oxidized (reduced), some product will be present and both oxidation and reduction processes can now take place.

When the sample contains only the reduced form of a redox couple (e.g., only HQ), the electrochemical process can be described by the voltammogram in Figure 4-3A.

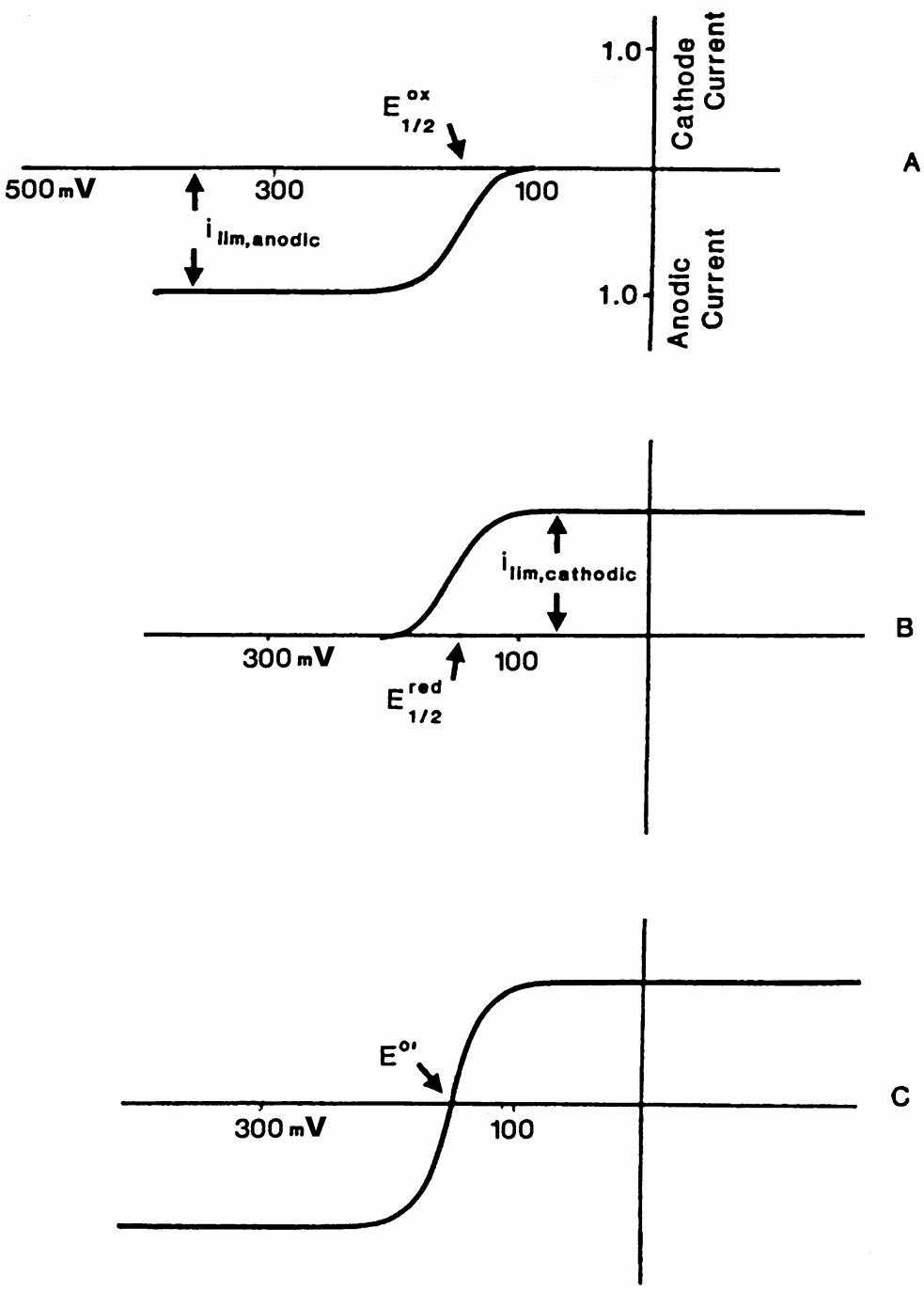

Figure 4-3: A - Reduced Form for the Current/Voltage Curves,
B - Oxidized Form of a Redox Couple,
C - The Combined Curve is the Combination of the Oxidized Form and the Reduced Form

This voltammogram indicates that:

a) At potentials that are negative relative to E°, little or no oxidation occurs.

b) At potentials that are positive relative to E°, the current will quickly reach its limiting value. Increasing the potential at that point will have essentially no additional effect on the current response obtained.

The equivalent voltammogram for the oxidized form of HQ is shown in Figure 4-3B. This curve is characterized by a region where no electrochemical activity will occur, as well as a region where the current has reached its limiting value. In a reversible electrochemical couple, the value of $E_{1/2}$ for the oxidation wave and $E_{1/2}$ for the reduction wave must be equivalent and correspond to E°'. $E_{1/2}$ is the potential at which the observed current is half that of the limiting value.

In Figure 4-3C, the voltammogram of an equimolar concentration of both forms of the redox couple is presented. Both an anodic and a cathodic limiting current can be observed. The potential where the current is zero is $E^{o'}$ (provided that the system is reversible and the diffusion characteristics of the two species are the same).

It should be noted that the "reversibility" of a redox couple is not a requirement for electrochemical detection. The critical factor is that the current must be proportional to the concentration (i.e., the process must be mass transport controlled).

In addition, the residual current (the current when no analyte is present) at the potential that is employed for the analysis must be small and stable, this latter requirement leads to a low noise level, increasing the signal to noise ratio and maximizing the sensitivity. While a reversible reaction is not required, a reversible reaction can be quite useful.

4.5 Coulometric vs. Amperometric Detection

There are two general types of electrochemical detectors:

- **Amperometric Detector**: When an amperometric detector is used, the eluent *flows by* the electrode surface. In this design, a fraction of the electroactive species in the eluent will be oxidized (reduced); but most of the electroactive compound flows by the electrode surface and does not react. The fraction of the electroactive compound that reacts is typically in the order of 5-15%.

- **Coulometric Detector:** When a coulometric detector is used, the eluent *flows through* a porous graphite electrode, rather than flowing by the electrode as in traditional electrochemical detectors. Since the surface area is large, essentially all of the electroactive species will be oxidized or reduced. Since a larger amount of the electroactive compound (10 to 20 times as much) is oxidized (or reduced) without a corresponding increase in noise, this detector can provide enhanced sensitivity. The current produced is directly related to the concentration of the species of interest via Faraday's law (Section 4.7).

Both amperometric and coulometric cells can be used with the ESA Coulochem III electrochemical detector to optimize performance for a given application. Coulometric detection is described in Chapter 5 and Amperometric detection is described in Chapter 6.

In most electrochemical analyses, the potential is held constant (DC mode) and the current is measured as a function of time. When an electroactive species flows through the cell, a current which is proportional to the concentration of the compound is generated. The fraction of the electroactive species that is converted at the electrode depends on a variety of factors; it should be noted that the coulometric electrode provides for essentially 100% conversion of the electroactive species.

4.6 Selectivity and Electrochemical Detection

If several electroactive species are present in a sample, the chromatographic separation is normally developed so that only one compound is presented to the detector at a given instant. If this is the case, the potential should be set to that value which provides the limiting current for the compound that is of primary interest.

If more than one compound in the sample is of interest and good chromatographic resolution is obtained, the potential can be set to the optimal potential of the compound requiring the highest potential.

If two (or more) electroactive compounds coeluted, the analyst should set the potential to optimize the current from the compound of interest while minimizing the current from the interferent. If, for example, the limiting current for a compound of interest is observed at 550 mV, but a trace component that co-elutes with the primary compound had an oxidation potential of 775 mV, it may be necessary to use a slightly lower value for the potential.

The analyst can determine if more than one electroactive compound is eluting from the column at a given instant by obtaining a voltammogram of the eluent and comparing it to voltammograms of known pure compound(s). The eluent voltammogram will be the composite of the individual voltammograms for the various species in the sample (provided that there is sufficient potential applied to oxidize (or reduce) all species present). An example of this is shown in Figure 4-4. It is advisable to use the minimum detector potential that results in the limiting current of the compound of interest to ensure that the contribution from other species which have higher limiting potentials is minimized.

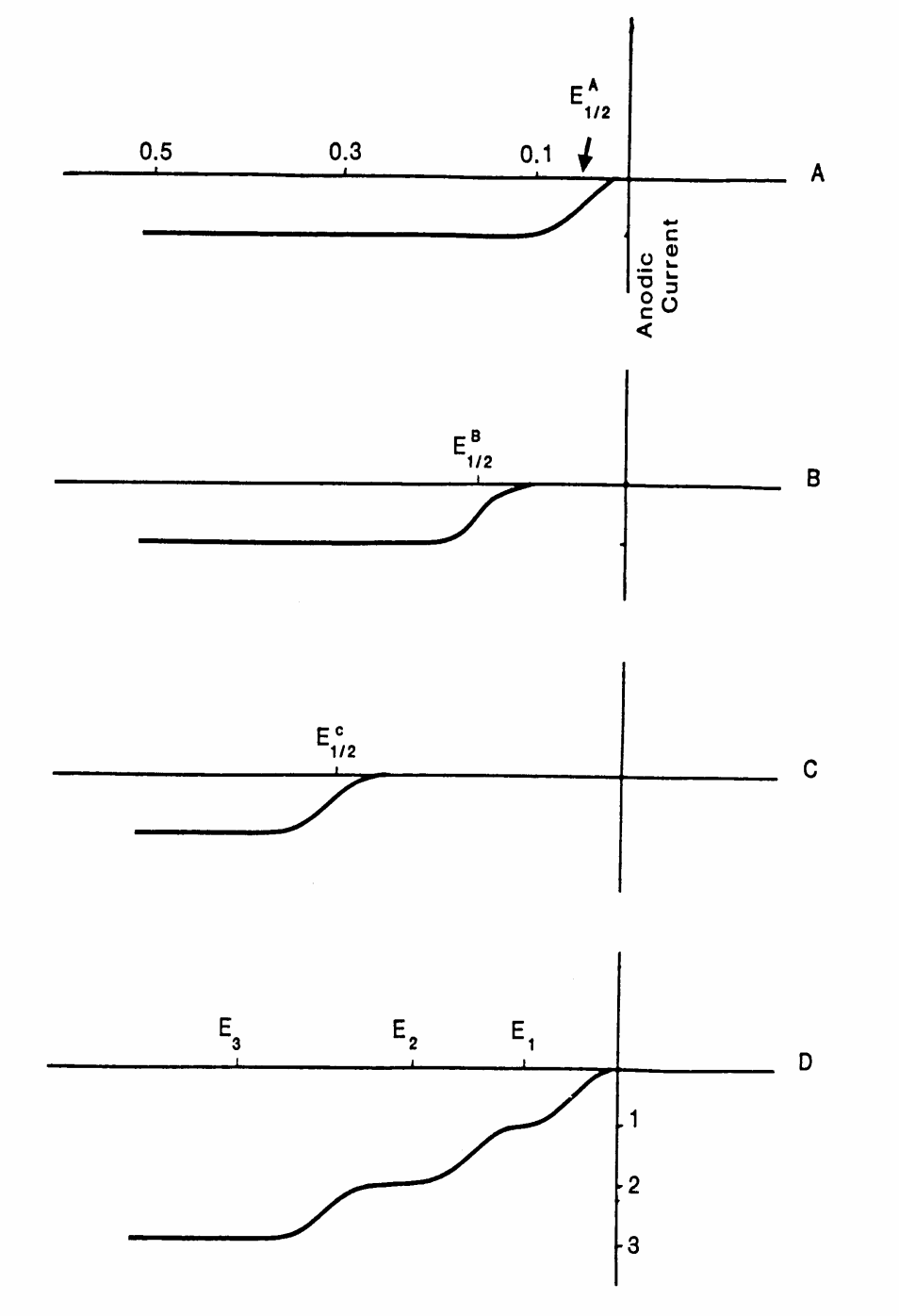

Figure 4-4: Current/Voltage Curves for a Three Component Mixture (for the sake of clarity, only the oxidation curves are presented) A, B and C Refer to the Individual Components, D is the Composite Curve for the Mixture

Additional selectivity can be obtained by using the first electrode in a dual channel coulometric cell as a screen electrode and the second electrode as the working electrode (Chapter 10). A screen electrode is set at a lower (or higher) potential than that required to oxidize (or reduce) the compound of interest so that interferences will be oxidized (reduced) at that electrode, but no response from the compound of interest will be observed.

When the detector is a coulometric detector, all co-eluting compounds which have an oxidation potential less than the compound of interest will be oxidized at the screen electrode (or reduced if they have a reduction potential which is less negative than the reduction potential of the compound of interest). This feature makes the use of the screen mode a powerful tool to enhance selectivity. Since amperometric cells oxidize only a small percentage of the analyte, it is not possible to use them in a screen mode to oxidize (or reduce) interferences.

4.7 Faraday's Law

Faraday's law (Equation 4-9) describes the charge that is generated by the oxidation (reduction) of a given quantity of reactant.

$$\mathbf{Q = (n)(F)(N)} \qquad \textbf{4-9}$$

where: **Q** is the total charge transferred (coulombs)
n is the number of electrons transferred (or the number of equivalents/mole)
F is Faraday's constant (96,500 coulombs/equivalent)
N is the number of moles of reactant

If the current is recorded as a function of time and the peak is integrated, Equation 4-9 can be presented as Equation 4-10:

$$\mathbf{Q = \int i\,dt = peak\ area} \qquad \textbf{4-10}$$

where: **i** is current (in amps)
t is time (in secs)

It can be shown that when an electrochemical LC detector operates with coulometric efficiency, the steady state current is defined by Equation 4-11:

$$\mathbf{i = (n)(F)(C)(v_f)} \qquad \textbf{4-11}$$

where: **i** is the current (in amps)
C is the concentration (moles/mL)
$\mathbf{v_f}$ is the flow rate (mL/sec)

4.8 Quantitation Using an Electrochemical Detector in the Coulometric Mode

When an electrochemical detector operates in a coulometric DC mode and the system uses a fixed potential, the observed cell current will fall at a rate that is proportional to the concentration of the analyte, as shown in Equation 4-12.

$$\textbf{Rate of electrolysis} = dC(t)/dt = -kC(t) \qquad \textbf{4-12}$$

where: **C(t)** is the instantaneous concentration of the analyte at a given time
k is the reaction rate constant (in electrochemistry, this is frequently called the cell constant)

Equation 4-12 describes a first order kinetic reaction. The integrated form of Equation 4-12 can be expressed in a variety of different (but equivalent) formats, as shown in Equations 4-13 through 4-15.

$$C(t) = C(0)\, e^{-kt} \qquad \textbf{4-13}$$

$$\ln (C(t)/C(0)) = -kt \qquad \textbf{4-14}$$

$$C(t)/C(0) = 2^{-t1/2} \qquad \textbf{4-15}$$

where: **C(0)** is the concentration at the onset of the electrolysis
$t_{1/2}$ is the number of half-lives that have elapsed since the onset of the electrolysis

When a coulometrically efficient electrode is employed, it is recommended that the peak area is used (rather than the peak height) for quantitation, since the peak area is directly related to the concentration of the compound of interest. If the flow rate changes, the peak height will change (but the area will not). The area under the curve is the integrated intensity of the coulometric signal, while the peak height is an instantaneous signal indicating the present current. The peak height will change with retention time, while the area does not.

Straightforward manipulation of Equations 4-13 through 4-15 allows for measurement of the degree of electrolysis as a function of the residence time of an analyte in the detector. It can be seen in Figure 4-5 that the detector can be operated with coulometric efficiency only if the cell constant (k) is large.

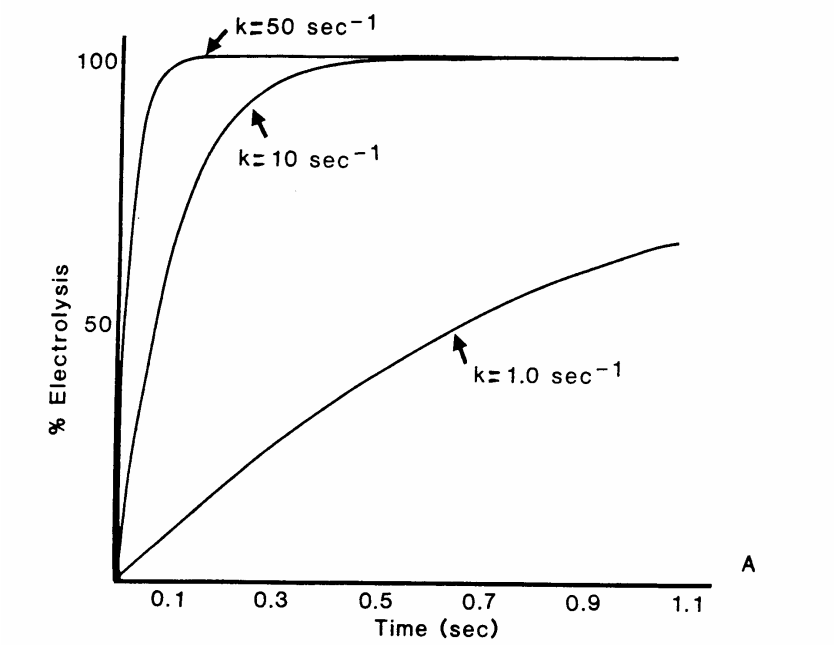

Figure 4-5: Efficiency of the Electrochemical Process with a Variety of Cell Constants

The cell constant is predominantly a factor of the mass transport properties of the cell, and can be determined by Equation 4-16.

$$\mathbf{k = (A)(D)/(V)(d)} \qquad \mathbf{4\text{-}16}$$

where: **A** is the surface area of the electrode (cm^2)
D is the diffusion coefficient of the analyte (cm^2/sec.)
V is the detector volume (cm^3)
d is the thickness of the diffusion layer (cm)

The diffusion layer represents the maximum distance a molecule can move into (or out of) the electrode surface during the measurement time. By fabricating the working electrode from a highly porous material with extremely small pores, d is minimized. In addition, the A/d ratio is maximized. The diffusion coefficient of typical analytes is in the order of 10^{-5} to 10^{-6} cm^2/sec and is dependent in part on the composition of the mobile phase (this is a factor which is not easily controllable).

Since the ESA Model 5010 Standard Analytical Cell (which is a coulometric cell) has a large area/volume ratio and the thickness of the diffusion layer is minimized, it has a large cell constant. A calculation of k, the "cell constant", based on a diffusion coefficient of 5 x 10^{-6} cm^2/sec for a typical analyte yields a cell constant of 500 sec^{-1}.

The large cell constant means that even if a significant portion of the cell surface is contaminated, the signal will not be reduced. In contrast, amperometric cells have a very small surface area and a correspondingly smaller cell constant. In Figure 4-6, the effect of contamination of the electrode on the electrode efficiency is presented. It can be seen that the ESA Model 5010 Standard Analytical Cell operates with coulometric efficiency for approximately 200 half-lives (a half-life is the period of time required for the response to fall by a factor of 1/2).

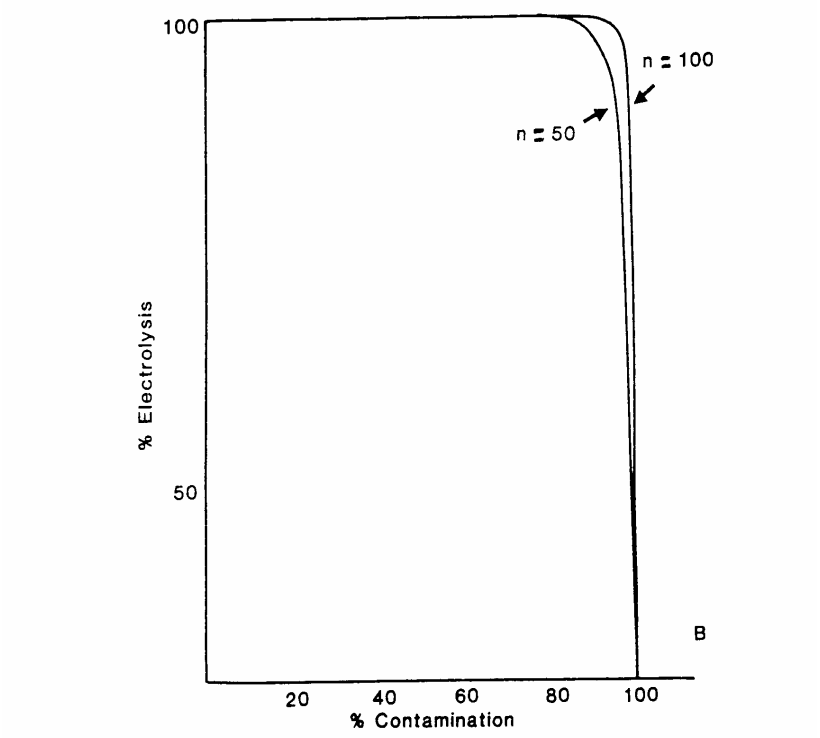

Figure 4-6: Efficiency of the Electrochemical Process as a Function of the Contamination of the Electrode at a Variety of Half Lives

A different way of expressing the effect of a large cell constant is in terms of the efficiency of the cell if it becomes contaminated. If, for example, 96% of the electrode surface in the ESA cell were to become contaminated, more than 99.6% of the electroactive species that passes through the cell would still be oxidized (or reduced). This means that the surface of the electrode in a coulometric cell is essentially maintenance free and has a longer useful lifetime before maintenance than electrodes in amperometric cells.

4.9 What Types of Compounds are Electroactive

A wide variety of compounds are capable of being monitored with an electrochemical detector. In general, electrochemical activity is dependent on the presence of an electroactive functional group. Many compounds, which are easily oxidized, contain groups with a lone pair of electrons (e.g., the hydroxy group of phenol or the amino group of aniline). Conversely, many compounds which are easily reduced contain groups such as a carbonyl or a nitro group.

The structures of some compounds that are easily oxidizable or reducible are shown in Figure 4-4. There are many classes of compounds which can be detected by electrochemical methods, including:

- Antioxidants: e.g., BHT, BHA, ND6A
- Carbohydrates: e.g., glucose, oligosaccharides
- Biogenic Amines and Metabolites: e.g., DA, NE, 5-HT
- Drugs of Abuse: e.g., LSD, morphine
- Enkephalin Peptides: e.g., methionine enkephalin
- Pesticides: e.g., aminocarb
- Pharmaceuticals: e.g., carbamazepine, fluorazepam
- Vitamins: e.g., ascorbic acid (vitamin C), vitamin K, tocopherols, carotenoids

In addition, many compounds can be readily converted into derivatives which are electroactive. A few examples of these techniques and applicable compounds include:

- Primary amino acids (pre-column derivatization with o-phthalaldehyde)
- Glycosides, acetylcholine (enzymatic methods)

There are a number of avenues available to determine if a compound is a potential candidate for electrochemical detection. These include:

- Searching the Literature: Computerized data bases can be readily used to see if electrochemical detection has been reported for the compound of interest.

- Exploratory Experimentation: A compound can be placed in the flow cell (i.e., injected into the HPLC without a column) with the detector set at a relatively high positive potential and a relatively high negative potential. If a response is observed, the compound may be electrochemically active.

- Manufacturer's Bibliography: ESA maintains a large listing of compounds for which electrochemical methods have been reported. Please feel free to contact ESA or your local representative for applications information.

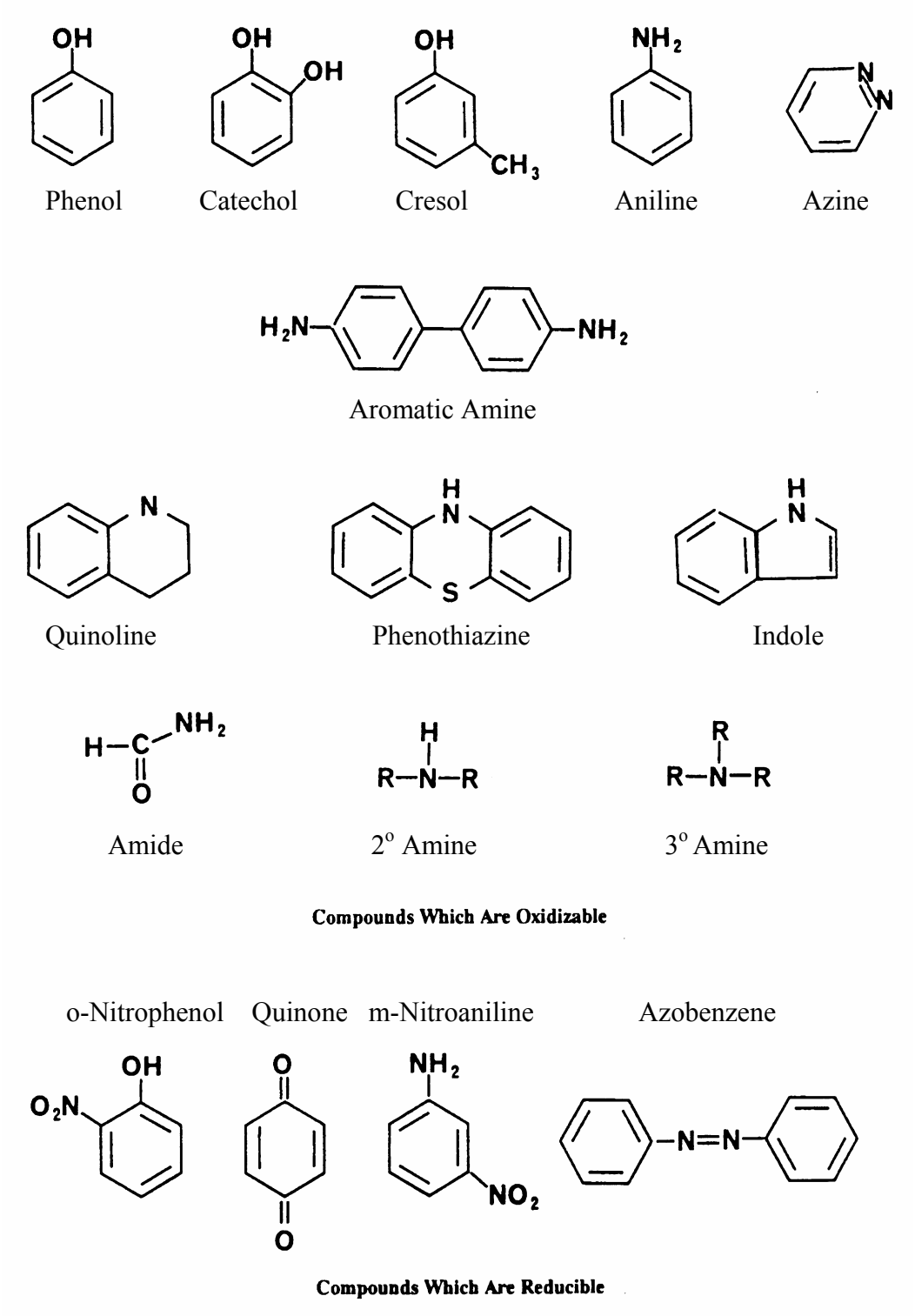

Figure 4-7: Typical Compounds Which are Electroactive

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5 The Coulometric Cell in Electrochemical Detection

5.1 Overview

The electrochemical cell is the component where the oxidation (reduction) occurs and the current from the oxidation (reduction) of the compound of interest is generated. This current is converted to an output voltage by the potentiostat, where it is processed and transmitted to the recording device. A short background of electrochemistry is presented in Chapter 4.

This chapter describes the coulometric cell and how it is used with the Coulochem® III to quantitate electroactive compounds. ESA provides a broad range of electrochemical cells for the Coulochem III, which are described in Section 1.3.2 of the *Coulochem III (50W) User's Guide Manual*. A discussion of the use and care of cells to maximize performance is presented in Section 5.5.

☞ NOTE: A discussion of the design and use of amperometric cells is presented in Chapter 6.

5.2 Design of the Coulometric Cell

A coulometric sensor involves the flow of the eluent *through* a porous graphite electrode as shown in Figure 5-1. The surface area of the electrode is very large, so that essentially all of the electroactive species will be oxidized or reduced (Figure 5-2). Since a much larger amount of the electroactive compound (10 to 20 times as much) is oxidized (or reduced) without a corresponding increase in noise, this detector can provide significantly greater sensitivity than an amperometric sensor. The ESA 501X Electrochemical Cells, the ESA 5020 Guard Cell and the ESA 5021 Conditioning Cell are coulometric sensors.

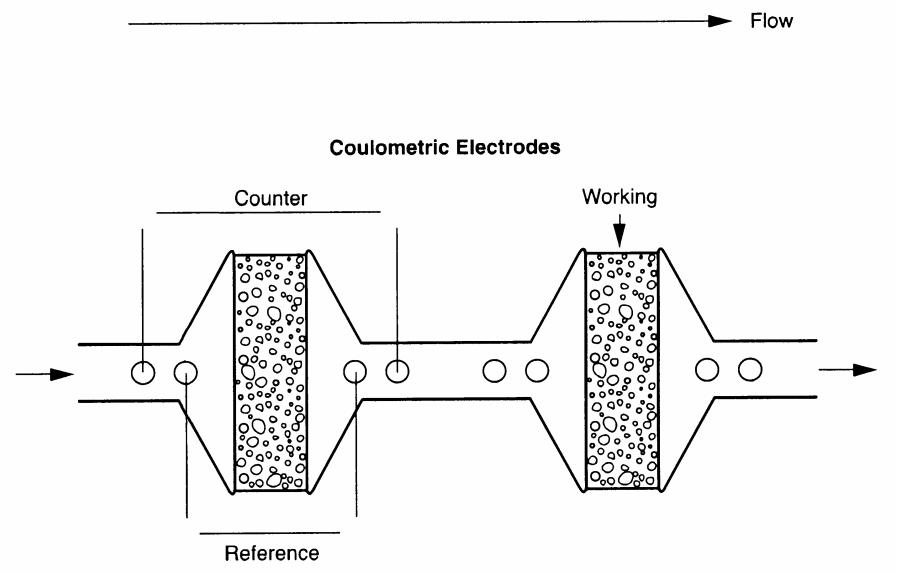

Figure 5-1: Cross-sectional View of a Coulometric Sensor

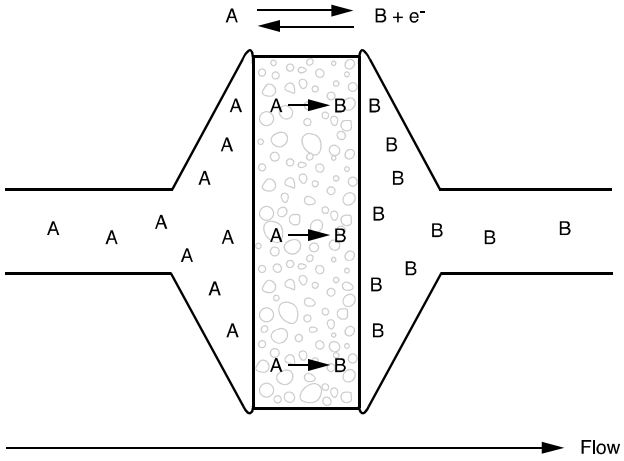

Figure 5-2: Efficiency of a Coulometric Sensor

In most applications involving coulometric sensors, the potential is held constant (DC mode) and the current is measured as a function of time. When an electroactive species flows through the cell; the current (which is proportional to the concentration of the compound that is oxidized/reduced) will increase/decrease with respect to the baseline. Since the current required to convert essentially all of the electroactive species is measured when coulometry is used, the area under the current/time curve can be integrated to obtain the number of coulombs that can then be directly related to the concentration according to Faraday's law (See Section 4.7).

5.3 Coulometric Detection and Selectivity

In some chromatographic separations, it may be very difficult or impossible to separate the sample so that only one compound is presented to the detector at a given instant. When this occurs, it may be possible to quantitate one or both compounds using two coulometric sensors, but it will not be possible to quantitate them if an amperometric sensor is used.

As an example of this point, lets consider the situation where two compounds co-eluted; one compound has an oxidation potential of 300mV and the other has an oxidation potential of 750 mV. If a single cell was used or if an amperometric sensor was used and the potential was set at 750 mV, the observed signal would be due to the oxidation of both compounds. On the other hand, if a potential of 300mV were used, the analyst could only quantitate the compound with the lower potential. This technique, which is termed Screen Mode or filter mode, is described in detail in Chapter 10.

5.4 Operational Benefits of the Coulometric Detector

There are several advantages of using a coulometrically efficient electrode for electrochemical analysis:

a) **The signal response is very stable**

The detector response remains constant even when 95% of the cell surface is rendered inactive by contamination or fouling. If an amperometric cell is used, any degree of surface fouling will lead to a decreased response. This decrease will be directly proportional to the loss in electrode surface activity.

b) **The signal size can be maximized**

When a coulometrically efficient cell is used, the signal from the sample can be maximized. Sensitivity in an analytical method is a function of the signal/noise ratio. To improve the sensitivity, one need only be concerned with minimizing the noise.

Better sensitivity leads to a lower “limit of detection” (LOD) for the assay. Alternatively, a more sensitive assay permits the use of a smaller sample, a concern when sample size is a critical issue. In some cases, this can eliminate the need for a pre-concentration step.

c) **The peak area and peak height for a sample are predictable**

When setting up a chromatographic analysis with a coulometric detector, it is often possible to predict the peak area and peak height for a given sample. This saves considerable time since the ordinate scale of the recorder can immediately be set properly (i.e., it is not necessary to make test injections to set the appropriate expansion factor).

An additional benefit of being able to predict peak height and peak area is the ability to identify peaks in a chromatogram. If several peaks are observed in a chromatogram, the knowledge of the peak area (or peak height) for a given compound can assist in assigning a given peak to that compound. This approach is commonly used in conjunction with the use of analytical standards to identify peaks in a chromatogram.

To predict the peak area, Equation 5-1 is used:

Peak Area = (W)(F)(n)/(MW) **5-1**

where: **W** is the weight (in g) of the compound of interest injected (not the weight of the sample)
n is the number of electrons transferred per molecule (equivalents/mole) [frequently 2 for many organic compounds]
MW is the molecular weight of the compound (g/equivalent)
F is Faraday's constant (96,500 coulombs/equivalent)

It is important to note that chromatographic variables are not required for this calculation, nor is it necessary to know the response factor of the detector. Equation 5-2 can be used to determine the peak height for a given compound; this equation reports the signal in amperes (and is based on the assumption that the peak is Gaussian).

Peak Height = (2)(A)/(W_b) **5-2**

where: **A** is the peak area (in coulombs)
W_b is the peak width at the base (in seconds)

To show the utility of Equations 5-1 and 5-2, consider the oxidation of hydroquinone (molecular weight is 110.11 g/mole) with a 2-electron transfer occurs. The peak area for a 10 ng sample can be found by Equation 5-3.

Peak area = (10)(96,500)(2)/110.11 = 17,528 nC **5-3**

If the peak base width is 30 sec, the peak height is expected to be 1,168 nA.

To determine the peak height that will be observed on the recorder, it is necessary to know the full-scale recorder range. To determine the percentage of recorder full scale, equation 5-4 is used.

Peak Height = (PH/FS) x 100 **5-4**
(% full scale)

where: **PH** is the peak height (in nanoamperes)
FS is the recorder full scale (in nanoamperes)

If, for example, the full-scale recorder range corresponds to 5000 nA, the peak will be 23.0% of recorder full scale.

Calculations of this type can be very helpful in method development and in the interpretation of chromatographic data. It should be noted that there are some approximations in this approach (i.e., the peak is perfectly Gaussian, the recorder is properly calibrated, there are no other electrochemical reactions, etc.). As an example of the utility of this approach, if the peak for a given compound is twice as large as is expected, one might conclude that something is co-eluting with the compound of interest or an incorrect peak assignment has been made.

d) **Coulometric Detection can provide additional selectivity**

A coulometrically efficient electrode can provide quantitative electrochemical conversion via the screen mode (Chapter 10) and Redox mode (Chapter 11). These modes of analysis allow for the use of a three-electrode system (using two cells: a conditioning cell and an analytical cell) to provide additional selectivity that may assist in differentiating between co-eluting species. In the Redox mode, additional selectivity (and sensitivity) can be obtained by reducing an electroactive species and then oxidizing the product (or vice versa).

e) **Coulometric Detection can provide a better response for irreversible analytes**

Many analytes of interest are oxidized (reduced) via an irreversible electrochemical process. This means that the current/voltage curve is broad and drawn out for amperometric EC detectors as shown in curve B of Figure 5-3.

However, most irreversible analytes have C/V curves that are essentially indistinguishable from reversible analytes on coulometric electrodes (see Figure 5-3, Curve A). This is because of the very large surface area of the coulometric electrode.

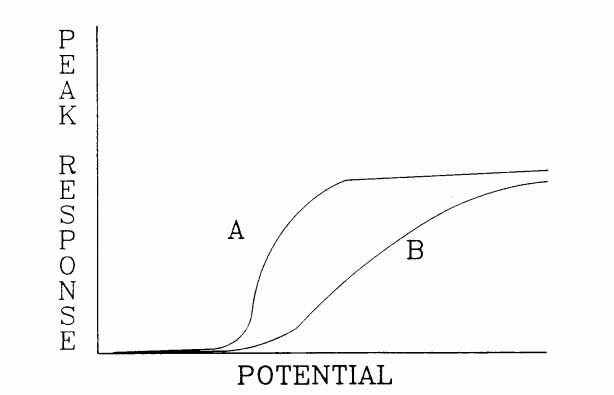

Figure 5-3: C/V Curve for an Irreversible Analyte for Both a Coulometric and an Amperometric EC Detector

A sharp C/V curve is desirable for two reasons:

1) A coulometric detector can be operated at a significantly lower potential for an irreversible analyte. This means that sensitivity can be optimized and interferents (occurring at more extreme potentials) can be minimized.

2) The response from an irreversible analyte will be more stable on a coulometric electrode since a well-defined limiting current is usually present.

If a detector is operated at a potential on the rising portion of the C/V curve, the analyte's response is less stable since any change in the C/V curve of the detector will cause a change in the quantification of the analyte. Operating on the limiting current portion of a C/V curve provides a "buffer" so that small shifts in the C/V curve will have no effect on the quantification of the analyte.

5.5 Installation, Use and Maintenance of ESA Coulometric Cells

Installation of ESA electrochemical cells is described in detail in Section 3.3.3. ESA coulometric cells are designed to require a minimum of maintenance. Cell problems can minimized by making sure that:

- The sample is filtered before the separation
- The mobile phase is filtered before use with a 0.2 micron filter membrane
- The stationary phase is stable with respect to the mobile phase
- The overall system is kept clean
- Excessive potentials are not applied to the cell
- The cell potential is turned off before the mobile phase flow is stopped

The cells may adsorb analyte electrolysis product or fines from the HPLC column over a period of time. An inline filter must be installed before the cell to ensure that particulate matter does not enter the cell, as particulate matter may clog the cell and lower system performance and/or create backpressure. The warranty for ESA cells is voided if an in-line filter is not placed immediately preceding the cell.

⚠ CAUTION: The cells may be placed in a temperature controlled chamber. Make certain that the temperature of the chamber is kept below 45°C. Above that temperature, the cells may undergo irreversible changes which can lead to the need to replace the cell. Failure to do so could damage the cells.

ESA Technical Note 70-1989, entitled Maintaining Cell Performance for Coulometric Sensors (Models 5010, 5011, 5014B, 5020, 5021, 6210) contains a detailed description of the use and maintenance of Coulometric cells. A copy of this note is supplied as Appendix D.

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6 The Amperometric Cell in Electrochemical Detection

6.1 Overview

The electrochemical cell is the component where the oxidation (reduction) occurs and the current from the oxidation (reduction) of the compound of interest is generated. This current is converted to an output voltage by the potentiostat, where it is processed and transmitted to the operating device. A short background of electrochemistry is presented in Chapter 4.

This chapter describes the amperometric cell and how it is used with the Coulochem® III to quantitate electroactive compounds. The ESA 5040 and 5041 cells are amperometric sensors that are described in detail in Section 1.3.2 of the *Coulochem III (50W) User's Guide Manual*.

NOTE: A discussion of the design and use of coulometric cells is presented in Chapter 5.

6.2 Design of the Amperometric Cell

An amperometric sensor involves the flow of the eluent *by* the working electrode surface as shown in Figure 6-1. In this design, some fraction of the electroactive species in the eluent will be oxidized (reduced); but most of the electroactive compound that flows past the electrode surface does not react. The amount of electroactive species that undergoes oxidation is dependent on the diffusion (mass transport) of the analyte to the electrode surface. The fraction of the electroactive compound that reacts is typically in the order of 5-15% at flow rates that are commonly used in HPLC (~1.0 mL/min); at lower flow rates, a larger fraction of the analyte can be oxidized (reduced). A cross sectional presentation of the amperometric sensor is presented in Figure 6-2, which shows that some, but not all of the analyte is oxidized (reduced).

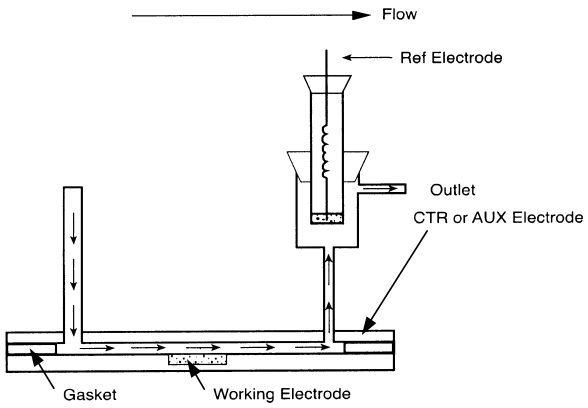

Figure 6-1: Cross-sectional View of an Amperometric Sensor

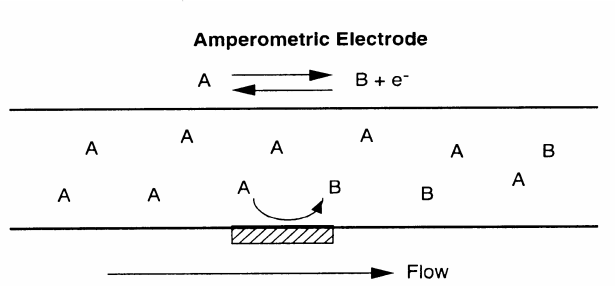

Figure 6-2: Efficiency of an Amperometric Sensor

6.3 Installation, Use and Maintenance of Amperometric Cells

Installation of ESA electrochemical cells is described in detail on Section 3.3.3. An in-line filter must be installed before each cell to ensure that particulate matter does not enter the cell, as particulate matter may clog the cell and lower system performance (e.g., create significant back pressure). The warranty for ESA cells is voided if an in-line filter is not placed immediately preceding the cells.

ESA amperometric cells are designed to require a minimum of maintenance. Cell problems can be minimized by making sure that:

- The sample is filtered before the separation
- The mobile phase is filtered before use with a 0.2 micron filter membrane
- The stationary phase is stable with respect to the mobile phase
- The overall system is kept clean
- Excessive potentials are not applied to the cell
- The cell potential is turned off before the mobile phase flow is stopped

⚠ CAUTION: The cells may be placed in a temperature-controlled chamber. Make certain that the temperature of the chamber is kept below 45°C. Above that temperature, the cell may undergo irreversible changes which can lead to the need to replace the cell.

Hints for Optimizing Operation

a) If it is necessary to remove the cell from the system, take care that the cell is not allowed to dry out. The cells should be rinsed to remove buffer, filled with Methanol/Water (50/50) and capped.

b) Always remove the potential from the cell (turn cells off) before removing the cell or stopping mobile phase flow.

c) From time to time, it may be necessary to clean the cells. A series of cleaning procedures is presented in Section 6.4.

d) In normal operation, mobile phase flow should be maintained at all times. Temporary and long-term shutdown procedures are described in Section 3.3 of the *Coulochem III (50W) User's Guide Manual*.

e) Ensure that all samples and aqueous components of the mobile phase are filtered through a 0.2 µm filter before use.

6.4 Cleaning the Target Electrode of the Model 5040/5041 Cell

From time to time, it may be necessary to polish the Target Electrode of the Model 5040/5041 Cell. A cross sectional view of the cell is shown in Figure 6-3.

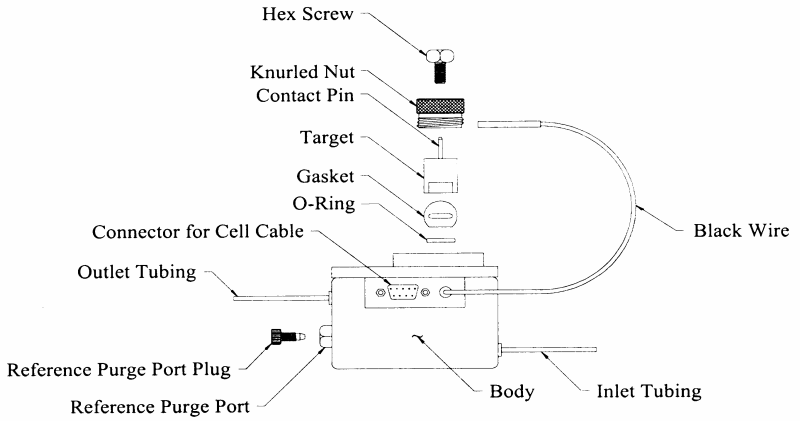

Figure 6-3: Model 5041 Amperometric Cell

A polishing kit (Part Number 56-0181) contains the necessary components to clean the target electrode (Table 6-1).

Table 6-1: Model 5040/5041 Cell Polishing Kit

Item	Quantity
0.3 µm Alumina Polishing Compound	1
Glass Plate	1
Nylon Polishing Discs	1
Microcloth Polishing Discs	1
600 grit Polishing Disks	1

⚠ CAUTION: Wear safety glasses and take any other needed safety precautions when polishing the target.

a) Fine Polishing

The need for fine polishing of the target is generally indicated by a gradual loss in detector output, tailing peaks, an increase in background current and/or an increase in baseline noise. Fine polishing is usually sufficient in order to restore prior performance.

☞ NOTE: The cell should be disconnected from the system when the target is removed and reinstalled. If this is not done, fluid may siphon into the cell and prevent a good seal.

⚠ CAUTION: Wear safety glasses and take any other needed safety precautions when polishing the target.

To fine polish the target:

1) Make sure that no potential is applied to the cell (the CELLS ON/OFF Activity indicator should not be lit).

2) Stop the flow through the cell and then disconnect the cell from the rest of the chromatographic system.

3) Disconnect the black wire that is connected to the target by gently pulling on the end of the connector as close to the target as possible.

4) Loosen the hex nut on the tip of the cell with a 3/8" wrench in a counterclockwise direction.

5) Turn the large knurled nut counterclockwise until the knurled nut is removed.

6) The round target assembly can now be removed from the cell by pulling it out of the cell body. Remove and discard the gasket using plastic tweezers, taking care that the counter electrode is not scratched. Do not re-use gaskets.

⚠ CAUTION: All internal parts of the cell should be handled carefully to prevent scratches and other damage, which may cause leaks.

7) Rinse the end of the target with purified water in order to remove any mobile phase that is adhering to the target surface.

☞ NOTE: In order to avoid the possibility of cross-contamination, do not use the same disk for polishing different types of targets (e.g., Gold and Platinum). Instead, use a separate disc for each target type and label each disk clearly for the type of target the be used on it.

8) Wet the microcloth with deionized water to make sure that the entire cloth is wet.

9) Shake the bottle of 0.3 µm Alumina before each use and add several drops of the polishing compound from the bottle to the microcloth disc.

10) Place the end of the target assembly (the end containing the gold, platinum or glassy carbon target element) flat on the disc and the polishing compound.

11) Spread the polishing compound around on the disk with the target and them polish the target by moving it around in a figure eight pattern on the disk for approximately one minute. Use only a moderate amount of pressure; otherwise the target will wear too quickly or unevenly.

☞ NOTE: The polishing disks are reusable and do not need to be rinsed after each polishing. After they are used for the first time, add only the needed amount of polishing compound and purified water to re-wet the disc.

12) After the target has been polished sufficiently (as noted by the absence of any dark color and the appearance of a smooth surface) rinse the target with purified water until all traces of the polishing compound have been removed. In addition, the target can be cleaned by placing it in water in an ultrasonic cleaner for 30-60 seconds.

13) Dry the target with a clean laboratory tissue (be careful not to leave any fingerprints on the target element).

14) Carefully dry the cavity that the target fits into with a tissue.

☞ NOTE: It is very important that the cell body is completely dry before reassembly.

To reassemble the cell:

1) Install a new gasket, ensuring that it is seated properly at the bottom of the cavity and that the o-ring is in position.

2) Carefully place the target back into the cell body cavity. The flat spot on the target should be aligned with the flat spot in the cavity and the target should contact the gasket at the bottom of the cavity.

3) Make sure that the hex nut has been sufficiently loosened in the knurled nut. Replace the knurled nut and tighten the nut (clockwise) until the nut just makes contact with the bottom of the bore, then back out ¼ to ½ turn and finger tighten the hex nut.

4) Using the supplied torque wrench, slowly tighten until you feel the wrench "click".

5) Reconnect the black wire to the target.

⚠ CAUTION: Do not over-tighten the hex nut as this may cause high background currents or damage the cell. Always use the torque wrench (Part Number 70-1713).

6) For a Model 5041 cell, reconnect the cell to the chromatographic system, purge the reference port, start the flow and then apply the proper potential. Check for leaks in the cell after starting the flow and occasionally during use. If a leak appears, turn the cell potential off, stop the flow, remove the target, rinse and dry the cavity and then reassemble the cell as above.

⚠ CAUTION: The Model 5041 cell must be purged before the potential is applied.

b) Coarse Polishing

Coarse polishing is to be used when the fine polishing procedure is not sufficient to remove the imperfection and produce a satisfactory surface. It uses the same procedure as the fine polishing protocol but a Nylon disk is used instead of the Microcloth disk.

c) Additional Cleaning Procedures

Occasionally the counter electrode in the analytical cell may need to be cleaned in order to obtain peak performance. If a dark thick coating has formed on the counter electrode (the stainless steel area at the bottom of the cavity in which the target is placed) then it should be removed. The presence of a light colored stain on the counter electrode is not detrimental to the performance of the cell.

To clean the counter electrode:

1) Carefully remove the gasket at the bottom of the cavity using tweezers. **Do not reuse the gasket.** Spare gaskets are supplied with the cell and additional gaskets can be ordered from ESA.

2) Use a dry cotton swab to scrub the discolored area of the counter electrode until all or nearly all of the discolored area is removed. If this is not sufficient to remove the deposit, a cotton swab moistened with a very small amount of polishing compound or a solvent such as methanol, isopropanol or acetone can be used to polish the counter electrode can be used. Be careful to avoid polishing over the inlet hole or outlet hole or o-ring to minimize the possibility of the polishing compound clogging these holes or adhering to the o-ring.

3) Rinse the counter electrode thoroughly with purified water followed by methanol by holding the cell on its side (without the target electrode) over a sink in order to remove the entire polishing compound. Dry the cell cavity with a laboratory tissue. After the cell is reconnected to the chromatographic system and the flow has been started, observe the backpressure to be sure that the cell has not become clogged. In the unlikely event that the cell is clogged, try reversing the flow to the cell in order to remove the blockage.

4) Remove any pieces of foreign material from the cell cavity and carefully replace the gasket in the cavity making sure that the gasket is seated properly at the bottom of the cavity.

5) Carefully place the target back into the cell body cavity and make sure that the flat spot on the target is aligned with the flat spot in the cavity and that it contacts the gasket at the bottom of the cavity.

6) Make sure that the hex nut has been sufficiently loosened in the knurled nut. Replace the knurled nut and tighten the nut (clockwise) until the nut just makes contact with the bottom of the bore, back it out ¼ to ½ turn and then finger tighten the hex nut.

7) Using the supplied torque wrench, slowly tighten until you feel the wrench "click".

⚠ CAUTION: Do not over-tighten the hex nut as this may cause high background currents or damage the cell. Always use the torque wrench (Part Number 70-1713).

8) Reconnect the cell to the chromatographic system, and purge the reference port (Model 5041 only). Then start the flow, apply the proper potential and check for leaks.

☞ NOTE: If the electrode is damaged due to mishandling of the electrode (e.g., dropping it) or if large scratches or imperfections appear in the target assembly, they may be removed by using the 600-grit disk wet with purified water. This should be done very carefully since it may be possible to remove too much of the target material. This procedure should be used only when absolutely necessary and not on a routine basis.

7 Maintenance Activities and Replacing Components

7.1 Introduction

Optimum performance of the Coulochem® III electrochemical detector will be obtained when the user performs a series of routine maintenance activities on a periodic basis. This chapter provides:

- Maintenance (Section 7. 2)
- Cell maintenance (Section 7.3)
- Replacing system components (Section 7.4)
- Cleaning (Section 7.5)
- Establishing s system log (Section 7.6)

☞ NOTE: Detailed maintenance activities for the coulometric cell are presented in Chapter 5 and detailed maintenance activities for the amperometric cell are presented in Chapter 6.

When the detector is initially installed, the analyst should obtain a chromatogram for a well-defined sample before developing a new analytical procedure. This chromatogram can serve as a benchmark to be used to check the performance of the system. Similarly, if problems are observed in the use of a specific analytical procedure, it may be useful to use the standard sample to ensure that the chromatographic system is functioning properly.

The user is encouraged to maintain a log of all operations of the detector; maintenance activities and all observed problems should be entered into the log. A discussion of the log is provided in Section 7.4.

☞ NOTE: ESA provides Instrument Qualification and Performance services for the detector. Call ESA (800) 275-0102 or your local ESA representative for additional details.

7.2 Maintenance

7.2.1 Overview

While the ESA Coulochem III electrochemical detector requires little day-to-day maintenance, we recommend that:

- Samples should be free of particulate matter. Filtering through a 0.22 µm Nylon or PVDF membrane filter is one useful method. Filters should be checked to ensure that extractable materials are not present and they are compatible with all constituents of the sample.

- The mobile phase should be filtered through a 0.22 µm Nylon or PVDF membrane. Make certain that the filter is compatible with all constituents of the sample.

- ESA in-line graphite or PEEK™ filters are used before each cell or series of cells.

Since the Coulochem III electrochemical detector is used as a part of a chromatographic system and the output from the detector reflects the performance of the overall system, it is important to perform all maintenance procedures for each of the various components (e.g., the solvent delivery module, injector, etc.) on a routine basis. The user should refer to the operating manuals for each part of the system and perform the necessary activities on a periodic basis. ESA provides preventive maintenance services, please contact ESA or your local ESA representative for details.

7.2.2 Daily Maintenance

☞ NOTE: The frequency for doing the various activities is dependent on the sample type, mobile phase composition, sample cleanliness and a number of other factors. The frequency indicated below should be considered as a guideline. As the user gains experience with the system and the analytical procedure, it is likely that a user-generated protocol will be developed.

On a daily basis (or every time that the unit is started up):

a) Check that the pump is working properly and the solvent bottle(s) contain sufficient mobile phase for the expected analysis.

b) There is sufficient pump seal wash solution.

c) The pump seal wash system is primed and flowing properly.

d) All connections are leak free. Check for the presence of salt on joints and the base of all components. If a salt deposit or leak is observed tighten the offending joint (but do not overtighten; if necessary make a new fitting).

e) If an autosampler is in the system, check that the tray temperature is correctly set, the syringe is bubble free and the wash syringe has sufficient wash solution for the day's analyses.

f) The filters in the solvent bottle and the mobile phase do not include any particulate matter. Replace solvent filters if they are discolored (the mobile phase should be filtered with a 0.2 micron filter membrane).

g) Run a test run using a standard or a well-defined sample and ensure that the background currents and peak heights (areas) have not changed appreciably from day to day.

h) Monitor the pressure and the temperature of the Thermal Organizer Module (if the latter is installed) and ensure that they have not changed significantly from the level that was observed on the previous day.

7.2.3 Weekly Maintenance

On a weekly basis:

a) Replace the filter element between the pump and the column (see Section 7.4.1).

b) Replace the pump washing solution.

c) Perform a flow rate check on the pump.

d) Check all electrical connections to ensure that they are properly seated.

e) Recalibrate the zero by performing an autozero with the power removed from the cells (CELLS OFF).

f) Perform all of the daily activities

7.2.4 Monthly Maintenance

On a monthly basis:

a) Inspect the condition of the PEEK tubing to detect potential problems and replace if necessary.

b) Perform all of the daily and weekly activities.

7.2.5 Quarterly Maintenance

On a quarterly basis:

a) Inspect and change the seals, check valves and pistons in the solvent delivery system (if necessary).

b) Replace the 10 µm mobile phase filters.

c) Replace the filter element between the column and the cells.

d) Perform all of the daily weekly and monthly activities.

7.3 Cell Maintenance

The cells may adsorb analyte, electrolysis products or fines from the HPLC column over a period of time. This process will slowly reduce the efficiency of the cells and can be minimized by ensuring that:

- The sample is filtered before injection.
- The mobile phase is filtered before use with a 0.2 micron filter membrane.
- The stationary phase is stable with regard to the mobile phase.
- The overall system is kept clean.

Removal of extraneous materials can frequently be performed by flushing the system with a solvent such as methanol or acetonitrile (the selection of the solvent will depend on the nature of the sample).

Additional information about cell maintenance can be found in Section 5.5 (Coulometric Cells) and Section 6.3 (Amperometric Cells).

7.4 Replacing System Components

7.4.1 Filter Elements

a) Frequency of Changing Filter Elements

In typical use, the filter elements become clogged and must be replaced on a periodic basis. The frequency of replacement is dependent on the level of particulate matter present in the mobile phase and the sample, as well as the production of fine particles from the analytical and/or guard column.

If the filter must be replaced very frequently (e.g., more than once a week) it may be worthwhile to modify the composition of the mobile phase and/or switch to a more stable column (e.g., a column from a different manufacturer), which might create fewer fines.

Microbial growth may occur in mobile phases with low levels (<3%) of organic solvents unless suitable precautions are taken.

A daily log of system pressure should be kept so that any pressure fluctuations can be monitored, as this is a good indication of clogged filter elements.

b) Checking the Pressure Drop Across the Filter

To measure the pressure drop across the filter element:

1) Remove the potential from the cells and turn off the mobile phase flow. Allow system pressure to drop to zero before disconnecting any components.

2) Disconnect the tubing on the downstream (outlet) side of the suspect filter.

⚠ CAUTION: Do not remove the system pressure by opening a fitting on the high-pressure side of the column. The rapid pressure drop can damage various components in the overall system. Always allow the system to drop to zero before breaking any connections.

3) Run the pump at a flow rate of 1 mL/min and record the pressure reading.

4) Disconnect the suspect filter and again record the pressure reading using a flow rate of 1 mL/min.

5) Determine the filter pressure drop by subtracting the second reading from the first reading. If the pressure drop is significant (100 psi [8 bar] or more), change the filter.

c) Changing a Filter

To change a filter:

1) Remove the potential from the cells and turn off the mobile phase flow. Allow system pressure to drop to zero before disconnecting any components.

⚠ CAUTION: Do not remove the system pressure by opening a fitting on the high-pressure side of the column. The rapid pressure drop can damage various components in the overall system.

2) Remove the filter assembly from the chromatographic system by removing the nuts on either end of the assembly.

3) Remove both end nuts from the filter assembly.

4) Remove the used filter. If necessary, *carefully* insert a small wooden dowel or plastic rod to dislodge the filter.

⚠ CAUTION: Be careful not to scratch the filter.

5) Rinse the filter housing with deionized water.

6) Replace one end nut. Insert a new ESA filter element into the filter housing. Ensure that the element is properly centered and seated against the surface of the end nut.

7) Replace the second end nut and tighten carefully until contact between the cap and the filter is felt. The filter is properly installed if both end nuts are approximately an equal distance from the center of the filter housing. The fitting should be finger tight, do not use a wrench or pliers and **DO NOT** overtighten as this can crush the filter, rendering it useless.

8) Re-install the filter housing in the chromatographic system. Ensure that the direction of flow is as indicated on the filter housing.

☞ NOTE: Initially, only the upstream end of the filter should be attached to the HPLC system. Pump about 5 mL of the mobile phase through the filter to waste before attaching the downstream end of the filter to the cell (this step will serve to wash the filter and ensure that particulate matter does not enter the cell).

7.4.2 Battery Backed Real-Time Clock

The Coulochem III stores methods in RAM. The system includes a battery backup on the Logic Module to provide sufficient power to maintain the storage when the power is turned off.

If the clock fails to keep time, the battery embedded in the clock integrated circuit may be low or defective. This is not a user serviceable part, and you should call ESA Service Department (800) 275-0102 or your ESA representative for assistance.

7.4.3 Changing the Line Fuse

If the unit does not power up when the main power switch is turned on or if the display and LED's suddenly are no longer illuminated, it is possible that the fuse has blown.

⚠ WARNING: Disconnect the Coulochem III from line power before removing the cover from the power input module. For continued protection against the risk of fire, replace only with the same type and rating of fuse.

To replace a fuse:

a) Turn off the power and remove the power cord from the unit.

b) Remove the cover from the power input module using a small screwdriver or similar tool.

c) Remove the fuse block assembly.

Remove all the fuses and replace with new ones. The appropriate fuses are indicated in Table 7-1.

Table 7-1: Fuses for Coulochem III Coulometric Detector

Voltage	Power Supply Board	Fuse Rating	Part Number
250 V	Standard	1A	70-6666

d) Holding the assembly on the sides, slowly slide the assembly in pressing in the front until seated properly.

e) Return the cover to the power input module.

f) Check that the proper voltage setting is selected on the top of the power module. Reinstall the power cord and turn the unit back on. If the fuses blow again, contact ESA or its representative for service. Do **NOT** continue to replace fuses, as this could cause damage to the detector.

7.5 Cleaning

7.5.1 Cleaning the Coulochem III Detector Unit

The outside of the detector can be cleaned with a soft towel moistened with a mild detergent. This should be suitable for removing dust and fingerprints. Avoid getting any liquid inside the detector as this could damage the unit. Avoid using abrasive cleaners especially on the keypad and the display as this could cause scratches.

If it becomes necessary to disinfect the detector, a mild bleach solution can be used. However, do not allow the bleach solution to remain on the detector for more than a few minutes. The bleach solution should be wiped off with a damp towel.

After cleaning, the detector can be dried using a soft towel.

7.5.2 Cleaning the Coulochem Organizer and Thermal Organizer

Follow the general cleaning procedures as described above for the detector unit. However, it is more likely that the organizer module will become dirty due to spills and leaks of the mobile phase over time. This typically will leave a white crusty residue from the electrolytes or salts used in making the mobile phase sufficiently conductive for electrochemical detection. These salts are almost always soluble in water. However, it may take some time to dissolve the salt residues during cleaning. Therefore, it may be useful to gently scrape the worst of the salt deposits from the interior surface of the organizer using a wooden stick (such as a tongue depressor). The rest of the residue can be removed with a damp towel. It may also be necessary to remove the inner part of the organizer, the piece that contains the cell(s), column(s), etc. to facilitate cleaning in and around the pan and the components.

The outside of all the cell(s), column(s), tubing, pulse damper, etc. may be cleaned according to the above procedure.

⚠ CAUTION: When you are cleaning the Thermal Organizer Module, take care that the cleaning material does not get on the Temperature Probe, the Thermostat or the Heater Driver Board (located on the underside of the chassis plate) as this could damage the organizer.

Clean the manual injector according to the manufacturer's instructions. If it becomes necessary to disinfect the organizer, a mild bleach solution can be used on all the surfaces. However, do not allow the bleach solution to remain on the organizer for more than a few minutes. The bleach solution should be wiped off with a damp towel.

After cleaning, the organizer and its contents can be dried using a soft towel.

7.6 Establishing a System Log

A log that includes the usage and maintenance as well as any comments about operation of the system should be maintained. This log should include the date, time, technician's name, number of samples, any maintenance activities and any relevant user comments about the performance of the system.

A typical sample log is presented as Figure 7-1. If a prescribed sample log format is provided by your organization that can be used to capture the relevant information.

Date	Time	User Name	Number Samples	Sample Type	Maintenance Activities	General Comments
7-3-01	11:22 AM	Jones	19	Catechols	Daily activities	OK
7-7-01	1:55 PM	Davis	27	Compd 2319	Weekly activities	Leaky fitting on column (repaired)
7-7-01	11:33 PM	Davis	29	Compd 2301	Daily activities	OK
7-8-01	2:12 PM	Jones	21	Compd 2318	Daily activities	Significant peak tailing replaced column
7-8-01	11:19 PM	Wold	33	Compd 2367	Weekly activities	OK
7-9-01	4:28 PM	Wold	23	Compd 2400	Daily activities	OK

Figure 7-1: A Typical Log

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8 Troubleshooting

8.1 Introduction

Troubleshooting refers to the determination of the cause of an abnormal condition or abnormal results from the detector. The analyst should recognize that if a problem is observed, it might be due to the detector, the cells, the column, the solvent delivery system or to some other component of the system. A detailed discussion of troubleshooting activities is presented in Section 8.3.

When the detector is initially installed, the analyst should obtain a chromatogram of a well-defined sample before developing a new analytical procedure. This chromatogram can serve as a benchmark to be used to check the performance of the system. Similarly, if problems are observed in the use of a specific analytical procedure, it may be useful to use the standard sample to ensure that the chromatographic system is functioning properly.

The user is encouraged to maintain a log of all operations of the detector; maintenance activities and all observed problems should be entered into the log. A discussion of the log is provided in Section 8.4.

8.2 Internal Electronic Diagnostic Programs

The Coulochem® III electrochemical detector includes an internal diagnostic test program that is used when the unit is powered up. This program is designed to determine if the electronics are functioning properly. If no faults are detected, the display will present the standard program screen. If the diagnostics program determines that there is an electronic fault, a message will appear on the display.

If a fault message appears on the display, turn the detector off, wait for a minute or two and turn the system on again. If the fault message re-appears, call the ESA Service Department at (800)-275-0102 or its representative. Please have the serial number of the instrument, the full text of the error message and the software version number available for the service engineer.

8.3 Troubleshooting Guidelines

8.3.1 Overview

Although an HPLC system with electrochemical detection consists of several components, troubleshooting can be simplified by consideration of the following guidelines:

a) In almost all cases, there is one proximate cause for the problem. As an example, if an increase in the baseline noise is observed, the problem can be caused by one of the following:

- The pump (e.g., the pump is not primed)
- The mobile phase (the mobile phase is not suitably degassed)
- The column (the column is contaminated and strongly eluted compounds are being eluted)
- The detector (there is an electronic problem)
- The cell may be contaminated
- A fitting (a fitting may be leaking)

b) A fundamental knowledge of the role of each component of the system is extremely useful in diagnosing the problem.

c) The availability of spare parts to substitute is very useful in diagnosing the problem.

d) If a problem is observed, run a “standard” sample to determine if the problem is instrument related or analysis related.

e) If any aspects of the analytical conditions is to be changed, run a “before” and “after” to ensure that the effect of the change is well understood. Do not consider any change as “trivial”. As an example, if you change the supplier of the buffer salt, verify that the change has no effect on the analysis.

f) To isolate the source of the problem, it may be valuable to perform independent checks of each of the components in the HPLC system. These tests should be found in the operator's manual for the individual components (see Section 2.6 for testing the Coulochem III detector).

A series of diagnostic procedures is presented below that will assist in pinpointing the cause of the problem. Since some problems from the pump or column are observed via the detector, we include a detailed discussion of potential problems for a typical system which includes HPLC with electrochemical detection.

⚠ CAUTION: When you are troubleshooting the system, do not relieve the pressure by opening a fitting on the high-pressure side of the instrument (e.g., do not open the fitting between the pump and column). Instead, reduce the flow rate to zero and let the pressure fall slowly. Failure to do this could damage various components in the system.

If it is necessary to remove the cells from the system, make certain that the potential is removed from the cells and the input/output lines are capped so that the cells are not allowed to dry out.

Ensure that all drain lines and grounding wires are securely attached.

8.3.2 Erratic/Noisy Baseline

Cause	Comments	Solution
Dissolved gases in the pumphead	If dissolved gases come out of solution in the pumphead the flow rate will be variable. This will cause cyclic noise. The frequency of the pattern will increase as the flow rate is increased.	Sparge the mobile phase with He for a few minutes or degas it via an ultrasonic bath and reprime the pump. Use a vacuum degasser.
Dissolved gases in the detector cell	If dissolved gases come out of solution in the detector cell, sharp noise spikes will be observed.	Increase the flow rate for 30 min to remove gases. Use a vacuum degasser. Remove the cell from the system, flush with water and then with degassed MeOH and again with water.
Pump head problem	Check pump seals/check valves for wear or leaks.	Replace seals if worn. Replace check valves if necessary.
Leaks in the system	Check for leaks in system.	Tighten all fittings.

8.3.2 Erratic/Noisy Baseline (Cont.)

Cause	Comments	Solution
Detector output voltage not matched to the recorder	Review installation procedure.	Set voltages correctly.
Mobile phase not properly mixed		Stir mobile phase.
Pulse damper membrane rupture	Check for leaks. Use a pulse damper known to be good.	Replace membrane or pulse damper.
Contaminants eluting from column	Trace levels of organic compounds may be tightly retained by the column.	Remove column and see if problem exists. Clean the column and/or replace.
System not grounded	All components of the system must be connected to a common ground.	Check AC line receptacle - verify that you have a true ground. Ensure that all HPLC components share the same ground.
Cell problem		Clean cell. See Appendix C for details. Replace cell.
Electronics problem in detector unit or cell cable		Perform detector test (Sect. 2.6, *Coulochem III User's Guide*). If problem persists, contact Service at (800)-275-0102
Air in reference purge port (Model 5014B, 5041 cells)		Purge reference electrode.
Cell temperature not held constant	Temperature variation potentially due to problem in organizer or temperature change in laboratory.	Maintain cells at constant temperature. If the Thermal Organizer is included in the system, make sure that it is operating properly (see Chapter 14).

8.3.3 High Background Currents

This section describes situations where the background current has noticeably increased in a short period of time.

Cause	Comments	Solution
Electroactive impurities in the mobile phase	See Notes a, b.	Increase the potential by 50 to 100 mV. The steady state mobile phase current will increase significantly if a component of the mobile phase is being electrolyzed. If there is more than one detector in the system, set both to the same potential and observe the current. If the current from channel 1 is higher than the current from channel 2, mobile phase impurities are likely. To determine the source of potential impurities, use another source for each component of the mobile phase on a sequential basis. Reduce the potential (if possible).
Electroactive species eluting from the column	This may occur when a new mobile phase or column is used.	Allow the system to equilibrate for an hour with the new mobile phase or until the baseline is stable (overnight if the mobile phase contains an ion-pairing agent) and check the current again. Remove column and re-establish flow. If currents drop, clean or replace column.
Cells operating at high potentials	Use of high potentials can shorten cell life.	Use lower potentials if possible.

8.3.3 High Background Currents (Cont.)

Cause	Comments	Solution
Adsorbtion on the electrode	Some electrochemical reactions lead to products that are adsorbed on the surface of the electrode. This will produce a decreased current (and an increase in noise).	Reversing the potential may restore the performance of the electrode. Clean cell (see Chapter 5 [coulometric cells] or Chapter 6 [amperometric cells] for details). See Appendix C for details.
Leaking cell		Check for wetness around cell and tighten fittings or replace cell.
Contaminants leaching from system components		Check mobile phase reservoir filters, column end frits, replace if necessary. Passivate system components.

a. If possible, avoid using triethylamine and other organic amines as chromatographic modifiers as organic amines tend to contain electroactive impurities.

b. High background currents are frequently observed when a new bottle of a reagent is used, the mobile phase has been stored for a period of time or some other change in the analytical protocol (frequently unintentional) is made.

8.3.4 Increase in Back Pressure

Cause	Comments	Solution
Accumulation of particulates from the mobile phase or injected samples		Replace in-line filter elements (see Sect. 7.4). Ensure that the mobile phase and/or samples are filtered through a 0.22 µm Nylon or PVDF membrane filter. Use a mobile phase with a substantial fraction of an organic solvent to prevent bacterial growth. Use freshly prepared mobile phase. Bacterial growth in the mobile phase may lead to clogging of the filter. ESA provides a suitable microbiocide reagent (Part Number 70-1025).
Clogged injector or column		Isolate suspect component. Refer to manufacturer's cleaning directions or replace rotor seal and/or stator face on injector. Ensure that the mobile phase and sample are filtered before use.
Plugged tubing		Isolate plugged tubing and replace.
Clogged cell		Remove cell from system and check back pressure. Clean cell (see Chapter 5 [coulometric cells], Chapter 6 [amperometric cells] and Appendix C for details). Replace cell if necessary.
Column temperature has fallen	Temperature variation potentially due to problem in organizer or temperature change in laboratory.	Maintain column at constant temperature. If the Thermal Organizer is included in the system, make sure that it is operating properly (see Chapter 14).

8.3.5 Loss of Response

This section describes the abrupt loss of a peak or peaks from the chromatogram when using a set of analytical conditions which is known to provide a useful chromatogram.

Cause	Comments	Solution
Accidental change of a parameter on the recorder, chromatograph, detector		Check settings and verify that the *Cells On/Off* indicator is lit.
Compounds of interest not sufficiently stable	Some compounds will decompose as a function of time.	Check stability as a function of time, and prepare fresh standards. If necessary, change conditions. Use a cooled autosampler.
Shift in the optimum potential		Generate new current/voltage curve to optimize operating potential. Electrochemically condition the cell (see Chapter 5 [coulometric cells], Chapter 6 [amperometric cells] or Appendix C for details.).
Change in pH or mobile phase composition	Mobile phases should be freshly prepared.	Check mobile phase and prepare fresh phases.
Recorder or cells not connected properly		Check all cable connections.
The injector may be partially or fully clogged	Make sure that the sample and mobile phase are clean.	Clean the injector.
Adsorbtion or fouling of the electrode or counter electrode (Model 5040/5041)	Graphite and glassy carbon electrodes can adsorb materials from the mobile phase and samples to hinder electrochemical response.	Clean electrodes (see Chapter 5 [coulometric cells], Chapter 6 [amperometric cells] or Appendix C.
Detector unit malfunction		Turn off module and re-initialize. If error message reappears, contact service. Perform detector test (Chapter 2). If problem remains, contact service.
Column temperature not held constant. Retention times changed	Temperature variation potentially due to problem in organizer or temperature change in laboratory. Retention times changed.	Maintain column at constant temperature. If the Thermal Organizer is included, make sure that it is operating properly (see Chapter 14).

8.3.6 Inability to Autozero the Signal

Cause	Comments	Solution
High background signal on a very sensitive current range (background >10 x gain, e.g., >500µA on 50 nA full scale)		Reduce potential or use a mobile phase that provides a lower background (lower concentration of electroactive species). Increase the current range.
Autozeroing on a peak or the void signal		Autozero on a relatively flat section of the chromatogram.
Autozero on a very noisy signal		Reduce the noise or increase the current range.

8.3.7 Guard Cell Parameters and Screen are not Displayed

Cause	Comments	Solution
Guard cell is not connected to the detector before the detector was powered up		Turn off detector and reconnect guard cell and guard cell cable to detector and then power up again.

8.3.8 Detector Test Procedure

Section 2.6 of the *Coulochem III (50W) User's Guide Manual* includes a test procedure that is used to verify that the detector unit is operating properly. This test procedure uses simulator test loads for the cell and the Guard Cell so that the results that are obtained are independent of the analytical cells.

If the operator believes that the Coulochem III detector unit is not functioning properly, the test procedure should be used. A worksheet is provided in with the test procedure that should be completed and sent to the ESA Service Department or your local distributor to assist in diagnosing and solving any problem in the detector unit.

8.3.9 Thermal Organizer Problems

If the Thermal Organizer is not functioning properly (i.e., the set temperature is not reached or maintained), check to make sure that the Interface cable between the organizer and the detector is securely plugged into the Logic Module, Power Supply Module and the PC board on the underside of the chassis plate of the organizer.

In addition, make sure that:

- The Temperature Probe is placed all the way into the column clamp and secured with the set screw.

- The Column Clamp is flush mounted on the chassis plate and is securely fitted to the plate.

9 Programming the Detector for DC Timeline Operation

9.1 The Role of Timeline Programming

In DC mode (*Coulochem® III (50W) User's Guide Manual*, Chapter 2), the system reports the cell current using the parameters set in the method when the CELLS are turned on. The parameters are held constant during data acquisition.

The DC+TIMELINE mode feature allows the user to perform the following operations during a DC data acquisition:

- A potential can be placed on a cell or removed from a cell or the operating potential can be changed incrementally or to discrete values.
- The current range and filter settings can be changed.
- The detector can be autozeroed on the current range in use or on all current ranges.
- Five contact closures can be opened, closed, and/or opened again.
- A marker can be placed on the detector output.
- Indicate that the detector should wait for a signal from an external device to perform an action (e.g., autozeroing the detector).
- And the timeline can be made to automatically repeat itself for repetitive complex analyses.

When a timeline operation is programmed, the system will perform the desired operation (or wait for an external signal) at the indicated time. As an example, consider the case where the potential for monitoring the eluant from a given separation is 500 mV, but 800 mV is required to monitor a compound that elutes at 12.3 min. In this scenario, the original potential is 500 mV and the following sequence might be used:

a) at 11.00 min, the potential is set to 800 mV
b) at 11.50 min, the system is autozeroed
c) at 13.00 min, the potential is reset to 500 mV
d) at 13.30 min, the system is autozeroed

In addition, DC+TIMELINE mode allows the user to set up looping operations, which are described in Section 9.4. Looping operations can be used to generate an automated Current/Voltage Curve (C/V Curve) or Hydrodynamic Voltammogram (HDV), in which the current is monitored at a series of potentials to determine the optimum potential for an analyte. An example of a looping operation is presented in Section 9.4.

9.2 Establishing DC+Timeline Conditions

DC+Timeline mode programming involves setting the initial operating conditions as well as time based conditions. The initial conditions are set in the same manner as DC mode (which is described in detail in Section 2.4 of the *User's Guide Manual*). For the convenience of the operator, we present the entire sequence of steps used to generate a DC+Timeline method in this section.

To set DC+Timeline mode operations:

a) Access the MODE SELECTION screen (Figure 9-1), by pressing [EDIT] on the COULOCHEM MAIN MENU.

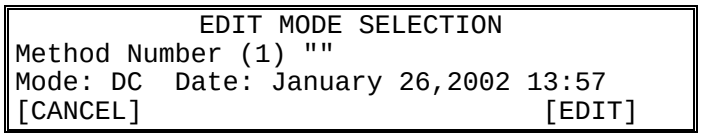


Figure 9-1: The Edit Mode Selection Screen

b) Press [EDIT] to access the MODE SELECTION screen (Figure 9-2). The cursor will be in the New Mode field.

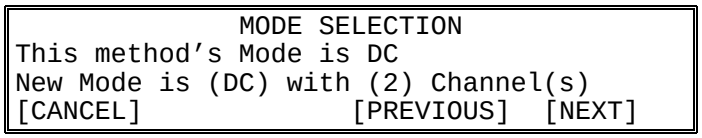


Figure 9-2: The Mode Selection Screen

c) Move the ▲or ▼key until DC+TL is indicated and press ENTER. The cursor will move to the *number of channels* field, which can be edited using the ▲or ▼key. If you change the *number of channels* field, press ENTER again. After the screen has been edited, press [NEXT] to the TIME DC MODE screen #1 (Figure 9-3).

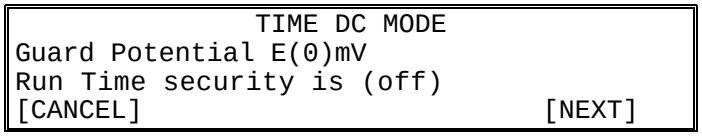


Figure 9-3: The Time DC Mode Screen #1

If a Guard Cell is not installed, the message *Guard Cell not detected* is presented and you should skip step (d).

d) Enter the desired *Guard Potential* using the numerical keypad (the range is between -2000 mV and + 2000 mV) and press ENTER. The value will be accepted and the cursor will move to the *Run time security* field. Typically, the Guard Cell potential is set at 25-50 mV above the highest analytical cell potential.

If an invalid value is entered for the *Guard Potential* (or any other parameter which is entered via the numerical keypad), the ENTRY LIMITS EXCEEDED screen (Figure 9-4) is presented. Press [NEXT] to return to the active screen.

```
          ENTRY LIMITS EXCEEDED
Value minimum: -2000,   maximum: 2000

                                  [NEXT]
```

Figure 9-4: The Entry Limits Exceeded Screen

e) If run time security is desired, ▲ or ▼ key should be pressed so that the field indicates **on**. When run time security is selected, a field to enter the security code is placed to the right of the *Run Time Security* field as shown in Figure 9-5. Security codes may be any one to four digit number except 0.

```
               TIME DC MODE
Guard Potential E (0)mV
Run Time Security is {on} code {0}
[CANCEL]                         [NEXT]
```

Figure 9-5: The Time DC Mode Screen #2

Press ENTER to activate the code field and enter a security code for this method. After you have entered the code, press ENTER and then press [NEXT] to access the TIME DC MODE CHANNEL 1 *Potential/Current Range* screen (Figure 9-6).

```
TIME DC MODE                CHANNEL 1
Potential E{50}mV
Current Range R (100uA)
[CANCEL]            [PREVIOUS]  [NEXT]
```

Figure 9-6: The Time DC Mode Channel 1 Screen #1

f) The *CH1 Potential/Current Range* screen is used to set the potential and current range for Channel 1. When the screen is accessed, the cursor will appear on the bracket to the right of the *Potential* field. Enter the desired potential using the numeric keypad (Range -2000 mV to +2000 mV). If an invalid entry is made, the ENTRY LIMITS EXCEEDED screen (Figure 9-4) will be presented. After the potential has been set, press ENTER to move the cursor to the *Current Range* field.

g) Select the desired current range via the ▲ or ▼ keys (the LED between the two keys will be illuminated). The range is from 10 pA to 1 mA in a series of 1-2-5 steps (10 pA, 20 pA, 50 pA, 100 pA, etc.). After the current range has been set, press NEXT to present an additional screen of parameters for TIME DC Channel 1 (Figure 9-7). The cursor will appear on the *Full scale output* field.

```
TIME DC MODE                CHANNEL 1
Full scale output: (1.0)Volts
Filter(5.0)Sec. Baseline Offset:{0}%
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 9-7: The Time DC Mode Channel 1 Screen #2

h) Select the desired *Full scale output* via the ▲ or ▼ keys (the choices are –1.0 V, -0.1 V, 0.1 V and 1.0 V) and press ENTER when the desired choice is made. The cursor will appear on the *Filter* field.

i) Select the desired filter via the ▲ or ▼ keys (the choices are 0.2 sec to 10 sec in a series of 1-2-5 steps) and press ENTER when the desired choice is made. The cursor will appear on the *Baseline Offset* field.

j) Select the desired baseline offset via the numeric keypad and press the [ENTER] key.

k) Press the [NEXT] key to present the TIME DC MODE CHANNEL 2 *Potential/Current Range* screen, which is similar to Figure 9-6, but describes the second DC channel. This screen is followed by the TIME DC MODE CHANNEL 2 *Full Scale Output/Filter/Baseline Offset* screen which is similar to Figure 9-7. Complete the two screens for DC TIME MODE Channel 2 in the same manner as for DC TIME MODE Channel 1. After you have completed the editing of the two screens, press [NEXT] to present the EDIT/CREATE TIMELINE EVENT screen (Figure 9-8), which is used to schedule events in the timeline.

```
        EDIT/CREATE TIMELINE EVENT
Time({0.00}) Min           Event (Cell)
Cell(s) (off)
[      ]   [ADD STEP]  [PREVIOUS] [NEXT]
```

Figure 9-8: The Edit/Create Timeline Event Screen

l) When Figure 9-8 is opened, the time when you want the event to occur can be entered. For Example, to key in a time of 1.53 minutes press the 1, then the 5, and then the 3 keys in the order given here. The numbers will move into the correct places after you have entered all of them. You must enter all times to two decimal places. After you have indicated the time, press ENTER to access the *Event* field. The ▲ and ▼ keys can be used to select the event that is to occur at the indicated time.

As you select an event, the third line of the screen changes to present the option(s) for that event. A summary of the various events and how they are edited is presented in Table 9-1.

Table 9-1: Timeline Event Options

Event	Data Entry Options	Method of Editing	Comments
Cells	On or Off	▲ and ▼ keys	This option is commonly used to turn cells off after a run. See Note b.
Range	Channel Number, Current Range	▲ and ▼ keys ▲ and ▼ keys	The current range for each channel can be set.
Set potl	Channel Number, Potential (E) mV	▲ and ▼ keys Numeric Keypad	The potential for each channel can be set. See Note b.
Add potential	Channel Number, Potential to Add E(0) mV	▲ and ▼ keys Numeric Keypad	The potential may be incremented for each channel during looping operations (see note b and Section 9.4).
Set contact	Select Contact (CC1) to CC5) Select On or Off or Toggle	▲ and ▼ keys ▲ and ▼ keys	External signal set on/off. Each contact closure can be set to on (closed) or off (open) or toggle (change state) as desired.
Hold for	Input Condition (Cell Off, Autozero, Start) Condition options are (off to on, on to off, off or on)	Cell Off, Autozero, Start ▲ and ▼ keys	Indicate what the system should wait for to perform the action.
Autozero	This will produce an autozero		See note a.
Marker channel	Marker Channel (1, 2 or 1&2) Height (-10 to +10)% FS	▲ and ▼ keys Numeric Keypad	Places a marker on the output.
Reset	This will reset to initial parameters		Contact closures will NOT be reset.
Loop End	Loop to (0.00) minutes, Number of Loops, (0-9999) This will stop the Timeline sequence	Numeric Keypad Numeric Keypad	Establish a set of repetitive operations. See Section 9.4 for details. Each Timeline must have an ending time.
Filter	Channel (1), Filter time (0.2 to 10.0)	▲ and ▼ keys ▲ and ▼ keys	Change the smoothing filter on the raw data.

a. Typically an autozero operation takes place before injection. If an autozero is performed during a run, it should be set at a point where the chromatogram is expected to be reasonably flat and the signal is low (i.e., the eluant does not contain electroactive compounds). For optimal performance, we suggest that a period of 15 seconds for a single range autozero and 35 seconds for a full autozero be provided between an autozero event and the onset of a peak.

b. For optimal performance, an AUTOZERO should be performed when a CURRENT RANGE change or a CELL POTENTIAL change is performed. If a data station is used, set the data station to inhibit integration during the period from 0.1 minutes before and after the gain range change.

A timeline can include a large number of events and each should be performed at a separate time. After you have edited or created a timeline step, you must press [ADD STEP] to present Figure 9-9, which confirms that the step has been saved. The [**SAVED**] label over the second soft key is presented to confirm that the step has been saved. Press the ENTER key to return the cursor to the Time parameter so that you may begin creating the next Timeline event. When you enter a time for another step, the bottom line returns to that of Figure 9-8.

```
        EDIT/CREATE TIMELINE EVENT
Time(10.00) Min            Event (Cell)
Cell(s) (off)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-9: The Edit/Create Timeline Event **SAVED Screen**

The display will then present a new event for editing. The [NEXT] key should be pressed after you have completed entering all steps in your timeline. To review/edit a previously entered step, use the ▲ and ▼ keys while the cursor is in the Time parameter field. In this manner you may quickly scroll back and forth through the times and events already entered.

m) When you select [NEXT], the SAVE METHOD screen (Figure 9-10) is used to store the method in memory. When the screen is opened, the cursor will be placed on the method number. You may scroll back through old methods already stored using the ▼key if you wish to overwrite an existing method. But you may not scroll past the first unused method number.

```
                SAVE METHOD
Save as method # (2)  {()}
New method mode : Undefined Date:----
[CANCEL]            [PREVIOUS]  [SAVE]
```

Figure 9-10: The Save Method Screen

n) Select the desired method number and press ENTER. The {( )} field will be activated and you can add a name to the method. Press a numeric key to enter a character, then press the ▲ or ▼ keys to select the desired character. After the number 9 is presented, alphabetic characters are presented so that you can enter names such as CAT2. The New Method Mode and the Date fields will be automatically filled in when the method is saved.

o) When you press SAVE, the method will be saved and a screen will be presented indicating that you should press the [NEXT] key to continue. This will present the COULOCHEM MAIN MENU.

9.3 Performing a DC+TIMELINE OPERATION

To Run a DC+Timeline Method:

a) Press [RUN] on the COULOCHEM MAIN MENU to present the RUN METHOD SELECTION screen (Figure 9-11).

```
          RUN METHOD SELECTION
Method Number (1)"ABC"
Type: DC+TimeLine  Date:Jan 5,2002 8:22
[CANCEL]                          [NEXT]
```

Figure 9-11: The Run Method Selection Screen

b) Use the ▲ or ▼ keys to select the desired method. When the desired method is presented, press [NEXT] to present the DC+TL METHOD RUNNING screen (Figure 9-11). This is the first of four screens that describe the operation of the DC+TL method (Figure 9-12 through Figure 9-15). The parameters indicated on this screen are described in Table 9-2 and can be edited as described below.

```
DC TL METHOD 2   RUNNING     TIME=0.00
E1{500}mV R1(1 mA) I1:-0.000mA  0%FS
E2{650}mV R2(1 mA) I2:-0.000mA  0%FS
[STOP]   [EVENTS]    [SETTINGS]  [GUARD]
```

Figure 9-12: The DC TL Method 2 Running Time Screen #1

☞ NOTE: The potential is automatically supplied to the cells when the [NEXT] key is pressed. Be sure there is mobile phase flowing through the cell before beginning a method.

Table 9-2: DC+TL Mode Parameters

Parameter	Description	Range	Default
Potential (E1, E2)	Voltage to be applied to the cell when cells are ON	-2000 mV to + 2000 mV (integer increments)	0 mV
Current Range (R1, R2)	Used to set the sensitivity level of the detector	100 pA to 1 mA (step increments)	1 mA
Current (I1, I2)	Instantaneous current	(not editable)	0

If desired, you can change the potential and the current range during a run. To edit a parameter, press the ENTER key until the cursor blinks at the desired parameter; set the desired value and press the ENTER key again. If the security feature was activated when this method was created, a SECURITY SCREEN will appear asking for the security code. If you do not enter the correct code, then the parameter you have changed will revert back to its old value.

NOTE: If you change a parameter in this manner, the change will be performed on an instantaneous basis, but will not be incorporated into the stored method. If you want to edit the method on a permanent basis, it will be necessary to edit the method as described in Section 9.2.

The [STOP] key terminates data collection.

NOTE: STOP does not turn the potential to the cell off, which must be done via the CELLS ON/OFF key, or inside the Timeline method.

The [EVENTS] key presents the DC METHOD RUNNING Screen #2 (Figure 9-15).

```
DC TL METHOD 2   RUNNING   12.34Min
Time (3.70)Min   Event: Cell
Cell(s) :Off
[STOP]   [CELL]   [SETTINGS]    [GUARD]
```

Figure 9-13: The DC TL Method 2 Running Time Screen #2

It is not possible to change or add steps to the timeline program while it is running. When the EVENTS screen is being displayed the cursor will be blinking to the right of the time entry field. You may scroll backwards and forwards through the timeline programmed steps to view them by using the ▲ or ▼ keys. But each step that is executed will not be displayed as it is executed or as it is about to be executed or just after it has been executed, etc.

c) The [SETTINGS] key accesses the DC METHOD Screen #3 (Figure 9-13), which displays the other DC method parameters status.

```
DC TL METHOD 2   RUNNING     12.34Min
Filt1(5.0)Sec  Output 1.0V  0%Offset
Filt2(5.0)Sec  Output 1.0V  0%Offset
[STOP]   [EVENTS]    [CELL]      [GUARD]
```

Figure 9-14: The DC TL Method 2 Running Time Screen #3

This screen displays the other standard DC parameters for each channel. These include for each channel the filter time constant (Filt), full scale output voltage (Output) and the Baseline Offset (%Offset) you have chosen for each channel for the method that is currently running. In this screen the filter time constants may be edited by pressing the ENTER key until the cursor is blinking next to the desired filter setting. After selecting a new filter setting press the ENTER key again to move to the next filter setting. After editing the second filter setting press the ENTER key once more to make the cursor disappear. New filter time constants are not implemented until the cursor is moved or disappears.

It is not possible to change the recorder output voltage or baseline offset while in RUN mode.

d) If you want to change the guard potential or view the present Guard Cell current, press [GUARD], which presents the DC TL METHOD RUNNING Screen #4 (Figure 9-15). Again change the potential value and press the ENTER key until the cursor stops blinking.

```
DC TL METHOD 2   RUNNING   TIME=12.34
Guard Potential: {0}mV
Guard Current: 1.23 mA
[STOP]   [EVENTS]    [SETTINGS]  [CELL]
```

Figure 9-15: The DC TL Method 2 Running Time Screen #4

e) Press the [CELL] key to return to the first DC Method Running screen where cell potentials, current ranges, currents, and recorder output signals are displayed.

9.4 Using Timeline to Generate Current Voltage Curves or Hydrodynamic Voltammograms

Successful operation of an electrochemical detector requires advanced knowledge of the optimum applied potential. This information is called a CV or HDV curve and one is usually generated for each analyte of interest. HPLC makes this task much simpler because mixtures of all the analytes of interest and their major impurities are separated before flowing through the EC detector. Since the generation of a CV curve is such an important part of method development we will create a timeline that performs that task as an example of the utility of timeline.

The two key timeline commands used in this effort are called *Loop* and *Add Potl.* Assume that your HPLC system consists of an autosampler, an integrator, isocratic pump, column, and the Model 5300 *Coulochem III* Electrochemical Detector. Assume also that all analytes and other peaks have eluted by nine minutes after injection and that you wish to generate a CV curve from -200 mV on the second electrode in your cell (T2) to +350 mV.

Connections

1. Connect the CC1 output contact closure on the *Coulochem III* to the input trigger on the autosampler such that closure of the contact will trigger an injection.

2. Connect the autosampler output contact closure to the recording integrator input such that the integrator will begin recording when the autosampler signals that an injection has been completed. Set the recording integrator input voltage to +1 volt and the recording time to 9.0 minutes.

***Coulochem III* Timeline Program Overview**

Time	Event	Device Identity	Device State	Comments
0.00	Set Contact	CC1	ON	Causes Injection
0.02	Set Contact	CC1	OFF	Resets CC1
10.00	Add Potl	CH2	+50 mV	Increments T2 Pot'l
12.00	Autozero		Rezero baseline after potential change	
12.50	Loop	Loop to 0.00 min	# of Loops = 10	Back to Inject
13.00	Set Pot'l	Channel 2	-200 mV	Resets T2 Pot'l
14.00	End	This will stop the time line sequence		

Creating this Method

a) Access the MODE SELECTION screen (Figure 9-16), by pressing EDIT on the COULOCHEM MAIN MENU.

```
           EDIT MODE SELECTION
Method Number (1) ""
Mode: DC   Date: January 26,2002 13:57
[CANCEL]                          [EDIT]
```

Figure 9-16: The Edit Mode Selection Screen

b) Press [EDIT] to access the MODE SELECTION screen (Figure 9-17). The cursor will be in the New Mode field.

```
              MODE SELECTION
This method's Mode is DC
New Mode is (DC) with (2) Channel(s)
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 9-17: The Mode Selection Screen

c) Move the ▲or ▼key until DC+TL is indicated and press ENTER. The cursor will move to the *number of channels* field, which can be edited using the ▲or ▼key. Change the *number of channels* to 2 and press ENTER again. Press the [NEXT] key to see the TIME DC MODE Guard Potential and Run Time Security Screen, Figure 9-17b.

```
               TIME DC MODE
Guard Potential E{0}mV
Run time security is {off}
[CANCEL]                          [NEXT]
```

Figure 9-17b: The Time DC Mode Screen

If you do not have a guard cell installed that will be noted. Assume we do not wish to run in secure mode.

d) Press the [NEXT] key to access the TIME DC MODE Channel 1 screen #1 (Figure 9-18).

```
TIME DC MODE               CHANNEL 1
Potential E{50}mV
Current Range R (100uA)
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 9-18: The Time DC Mode Channel 1 Screen #1

e) The *CH1 Potential/Current Range* screen is used to set the initial potential and initial current range for Channel 1. When the screen is accessed, the cursor will appear on the first character of the *Potential* field. Enter -200 mV using the numeric keypad. After the potential has been set, press ENTER to move the cursor to the *Current Range* field. Select the 10 µA current range via the ▲ or ▼ keys (the LED between the two keys will be illuminated). After the current range has been set, press [NEXT] to present an additional screen of parameters for TIME DC Channel 1 (Figure 9-19). The cursor will appear on the *Full scale output* field.

```
TIME DC MODE                CHANNEL 1
Full scale output: (1.0)Volts
Filter(5.0)Sec.  Baseline Offset: {0}%
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 9-19: The Time DC Mode Channel 1 Screen #2

f) Set the desired *Full scale output* via the ▲ or ▼ keys to 1 V and press ENTER when the desired choice is made. The cursor will appear on the *Filter* field.

g) Select a 2 second filter via the ▲ or ▼ keys and press ENTER when the desired choice is made. The cursor will appear on the *Baseline Offset* field.

h) Press the [NEXT] key to present the TIME DC MODE CHANNEL 2 *Potential/Current Range* screen, which is similar to Figure 9-6, but describes the second DC channel. Set *Potential* to -200 mV and *Current Range* to 10 µA. Press [NEXT] to reach the TIME DC MODE CHANNEL 2 *Full Scale Output/Filter/Baseline Offset* screen which is similar to Figure 9-19. Choose 1 V *Full Scale Output*, 2 second *Filter*, and 0% *Baseline Offset.* After you have completed the editing of the two screens, press [NEXT] to present the EDIT/CREATE TIMELINE EVENT screen (Figure 9-20), which is used to schedule events in the timeline.

```
        EDIT/CREATE TIMELINE EVENT
Time({0.00}) Min     Event (Set Contact)
Set Contact(CC1)     to (on)
[     ]  [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 9-20: The Edit/Create Timeline Event Screen

i) Press ENTER to accept the first time as (0.00) Min and to access the *Event* field. Use the ▲ key to display Set Contact. Press ENTER again to accept that the Event at 0.00 minutes will be to set a contact closure. Press ENTER again to accept that you wish to set CC1 (which is connected to the autosampler inject input). Use the ▲ key to set the state of CC1 to on.

At this point you have created the first line in your timeline program (See the Timeline Program Overview above). **HOWEVER you have not saved it.**

j) **You must remember to press the [ADD STEP]** key after every step is created. Press [ADD STEP]. You will see the Saved Screen, Figure 9-21.

```
        EDIT/CREATE TIMELINE EVENT
Time({0.00}) Min     Event(Set Contact)
Set Contact(CC1)     to (on)
[DELETE]  [**SAVED**]  [PREVIOUS] [NEXT]
```

Figure 9-21: The Edit/Create Timeline Event **SAVED Screen**

k) When the autosampler has finished it's injection sequence it will again check the status of it's inject command contact closure. By that time we must have opened the CC1 contact or another injection will occur. So our second timeline step must be to turn CC1 off again.

Press ENTER to see the following:

```
        EDIT/CREATE TIMELINE EVENT
Time({0.00}) Min     Event (Set Contact)
Set Contact(CC1)     to (on)
[     ]  [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 9-22: The Edit/Create Timeline Event Screen with Set Contact Event Already Presented

l) The cursor will be on the Time parameter. Press the 0 key twice and then press the 2 key. Press ENTER three times to reach the on/toggle/off parameter. Use the ▼ key to display off. You should now see:

```
        EDIT/CREATE TIMELINE EVENT
Time({0.02}) Min     Event (Set Contact)
Set Contact(CC1)     to (off)
[      ] [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 9-23: The Edit/Create Timeline Event Screen with Set Contact CC1 Turned OFF at 0.02 Minutes

m) Press [ADD STEP] to save, and then press ENTER to return to the Time Parameter.

n) In like manner create the **Saved** Timeline Event Screens shown below.

```
        EDIT/CREATE TIMELINE EVENT
Time({10.00}) Min    Event(Add Potl)
Channel(2)     Potential to add(50)mv
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

```
        EDIT/CREATE TIMELINE EVENT
Time({12.00}) Min    Event(Autozero)
This will produce an Autozero
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

```
        EDIT/CREATE TIMELINE EVENT
Time({12.50}) Min    Event(Loop)
Loop To (0.00) Number of Loops(10)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-24: The Saved Edit/Create Timeline Event Screens

As each loop occurs the Channel 2 potential will change from -200 mV to -150 mV to -100 mV to -50 mV to 0 mV to +50 mV and so on until it reaches +350 mV. An injection will occur and a chromatogram will be recorded at each Channel 2 potential. We allow two minutes for the baseline to stabilize after a potential change before autozeroing.

```
        EDIT/CREATE TIMELINE EVENT
Time({13.00}) Min    Event(Set Potl)
Channel(2)     Potential E(-200)mV
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-25: The Edit/Create Timeline Event Screen #1

Finally we reset the channel 2 potential back to its original value of -200 mV before stopping the experiment at 14.00 minutes. Please add the step at 13.00 minutes (shown above in Figure 9.25) and the final step at 14.00 minutes (shown below in Figure 9.26) to the timeline program you are creating.

```
        EDIT/CREATE TIMELINE EVENT
Time({14.00}) Min    Event(End)
This will Stop the time line sequence
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-26: The Edit/Create Timeline Event Screen #2

o) Before saving this method you should review the Timed Events portion. Press the ENTER key until the cursor is on the Time parameter. Now use the ▲ and ▼ keys to step through the times saved in the Timeline. As you step to each time the event programmed for that time will be displayed. When you are satisfied that your timeline program is correct, press the [NEXT] key to display the SAVE METHOD screen, Figure 9-24.

```
                SAVE METHOD
Save as method # (2) {()}
New method mode : Undefined Date:----
[CANCEL]             [PREVIOUS]  [SAVE]
```

Figure 9-27: The Save Method Screen

p) The cursor should be on the method number. Use the ▲ and ▼ keys to scroll to a method number that is unoccupied. In this case it's Method #2. Press the ENTER key to accept the Method number. The cursor will jump to another set of nested brackets on the same line. It is now possible to name your method.

q) To name your method CV.CURVE:

Press the 9 key and then the ▲ key until a C is showing
Press the 0 key and then the ▼ key until a V is showing
Press the 0 key and then the ▼ key until a "." is showing
Press the 9 key and then the ▲ key until a C is showing
Press the 0 key and then the ▼ key until a U is showing
Press the 0 key and then the ▼ key until an R is showing
Press the 0 key and then the ▼ key until a V is showing
Press the 9 key and then the ▲ key until an E is showing
Now press the ENTER key and then the [SAVE] key

Messages about the saving process will be displayed and finally you will see the METHOD STORED screen as depicted here as Figure 9-25.

```
               METHOD STORED
Saved as method 2
Select the NEXT key to continue
                                  [NEXT]
```

Figure 9-28: The Method Stored Screen

The [NEXT] key will return you to the COULOCHEM MAIN MENU.

r) Had we wished we could have also turned the cells off to protect them until we returned to check our results. Some pumps have a STOP FLOW contact closure. We could have wired CC2 to this and stopped mobile phase flow after turning the cell off before we ended the method. There are other options as well. Some of these will be explored in the next example, Changing Current Ranges.

s) NOTE: Occasionally after saving a timeline step the [NEXT] key is pressed instead of the ENTER key. When this happens you will exit the Edit/Create Timeline Event screen and be asked to save your method. Simply press the [PREVIOUS] key to get back to where you were when you inadvertently pressed [NEXT].

9.5 Using Timeline to Change Current Ranges Reproducibly During Each Analytical Chromatographic Run

In this second example assume that your chromatographic system is very simple. It consists of a pump, a manual injector with position sensing switch, a column, this detector, and a simple chromatographic recording peak integrator. Also assume that your samples are quite complex yet you have perfected a separation that isolates the peaks which you need to quantitate. The only problem is that the concentration of the analytes varies widely so you need to somehow change detector sensitivity in a reproducible fashion at various times during each chromatographic run.

Changing detector sensitivity at various times during development of each chromatogram requires precise timing and a knowledge of when sample injection has occurred. Assume that you have run a few chromatograms manually and now know that Peaks A, B, and C elute at 2.5, 4.5, and 6.5 minutes, respectively. You also know that probable concentration ranges will be such that 10 μA, 50 nA, and 500 nA detector current ranges are optimum for peaks A, B, and C, respectively and that a potential of +300 mV is all that is required to oxidize all three compounds.

Connections

1. Connect the CC1 output contact closure of the *Coulochem III* to the input trigger on the recording integrator such that closure of the contact will trigger data recording. Set recording integrator input range to 1 volt and recording time to 9.49 minutes.

2. Connect the *Coulochem III* DC Signal Out 2 BNC connector to the Data In terminals on the recording integrator.

3. Connect the injector position sensing leads to the START input contact closure of the *Coulochem III* so that every injection will trigger an automatic timed series of events.

***Coulochem III* Timeline Program Overview**

Time	Event	Device Identity	Device State	Comments
0.20	Autozero			
0.21	Autozero	Creates an initial all range autozero. Requires ~35 seconds.		
1.00	Hold For	Start	OFF to ON	Waits for sample injection.
1.01	Set Contact	CC1	ON	Starts Data Integrator
1.03	Set Contact	CC1	OFF	Resets CC1
4.50	Range	CH2	50 nA	Ready for Peak B
7.00	Range	CH2	500 nA	Ready for Peak C
10.50	Range	CH2	10 μA	Ready for Peak A
10.51	Autozero			
10.52	Autozero	Creates an all range autozero		
11.60	Loop	Loop to 1.00 min # of Loops = 1000		Back to Inject
121.00	End	This will stop the time line sequence		

Creating this Method

a) Access the MODE SELECTION screen (Figure 9-25), by pressing [EDIT] on the COULOCHEM MAIN MENU. And use the ▲ key to scroll up to an unused method.

```
           EDIT MODE SELECTION
Method Number (3) ""
Mode: DC  Date: January 26,2002 13:57
[CANCEL]                          [EDIT]
```

Figure 9-29: The Edit Mode Selection Screen

b) Press [EDIT] to access the MODE SELECTION screen (Figure 9-26). The cursor will be in the New Mode field.

```
              MODE SELECTION
This method's Mode is DC
New Mode is (DC) with (2) Channel(s)
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 9-30: The Mode Selection Screen

c) Press the ▲or ▼keys until DC+TL is indicated and press ENTER. The cursor will move to the *number of channels* field, which can be edited using the ▲or ▼key. Change the *number of channels* to 2 and press ENTER again. Press the [NEXT] key to see the TIME DC MODE Guard Potential and Run Time Security Screen, Figure 9-30b.

```
                TIME DC MODE
Guard Potential E{0}mV
Run time security is {off}
[CANCEL]                          [NEXT]
```

Figure 9-30b: The Time DC Mode Screen

If you do not have a guard cell installed that will be noted. Assume we do not wish to run in secure mode.

d) Press the [NEXT] key to see the TIME DC MODE CHANNEL 1 screen #1 (Figure 9-31).

```
TIME DC MODE                CHANNEL 1
Potential E{50}mV
Current Range R (100uA)
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 9-31: The Time DC Mode Channel 1 Screen #1

e) The *CH1 Potential/Current Range* screen is used to set the potential and current range for Channel 1. When the screen is accessed, the cursor will appear on the first character of the *Potential* field. Enter -200 mV using the numeric keypad. After the potential has been set, press ENTER to move the cursor to the *Current Range* field. Select the 10 µA current range via the ▲ or ▼ keys (the LED between the two keys will be illuminated). After the current range has been set, press [NEXT] to present an additional screen of parameters for TIME DC Channel 1 (Figure 9-32). The cursor will appear on the *Full scale output* field.

```
TIME DC MODE                CHANNEL 1
Full scale output: (1.0)Volts
Filter(5.0)Sec. Baseline Offset:{0}%
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 9-32: The Time DC Mode Channel 1 Screen #2

f) Select the desired *Full scale output* via the ▲ or ▼ keys to 1V or whatever the recording integrator full scale range is and press ENTER when the desired choice is made. The cursor will appear on the *Filter* field.

g) Select a 2 second filter via the ▲ or ▼ keys and press ENTER when the desired choice is made. The cursor will appear on the *Baseline Offset* field.

h) Press the [NEXT] key to present the TIME DC MODE CHANNEL 2 *Potential/Current Range* screen, which is similar to Figure 9-31, but describes the second DC channel. Set *Potential* to 300 mV and *Current Range* to 10 μA. Press [NEXT] to reach the TIME DC MODE CHANNEL 2 *Full Scale Output/Filter/Baseline Offset* screen which is similar to Figure 9-32. Choose 1 V *Full Scale Output*, 2 second *Filter*, and 0% *Baseline Offset.* After you have completed the editing of the two screens, press [NEXT] to present the EDIT/CREATE TIMELINE EVENT screen (Figure 9-33), which is used to schedule events in the timeline.

```
        EDIT/CREATE TIMELINE EVENT
Time({0.00}) Min    Event (Cell)
Cell(s) (off)
[      ] [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 9-33: The Edit/Create Timeline Event Screen

i) Press 20 to enter the first time as (0.20) min. Press ENTER to access the *Event* field. Use the ▲ key to display Autozero. Press ENTER again to accept Autozero at 0.20 minutes. This will move the cursor to the Input choice. Press ENTER again. The EDIT/CREATE TIMELINE EVENT screen should now look like Figure 9-34 below. Press [ADD STEP] to save this first step in the timeline.

```
        EDIT/CREATE TIMELINE EVENT
Time({0.20})Min      Event(Autozero)
This will produce an Autozero
[     ]  [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 9-34: The Edit/Create Timeline Event Screen with Autozero Event

Repeat the first program step by changing the time to 0.21 minutes and saving a second Autozero event. Two autozero commands issued within one second of each other will cause a full autozero on all current ranges, which is what is needed when you intend to change them during a chromatographic run.

Now create a situation where the timeline will pause until an injection is made. For simplicity change the time to 1.00 minutes by pressing the 1 key and then the 0 key twice. Press enter to move the cursor to the event field and then press the ▼ key to display the "Hold For" event. Again press enter to reach the input field and press the ▼ key to display "Start". Press enter to accept Start and access the condition field. Press the ▼ key until the condition "off to on" is displayed.

Press enter and then press the [ADD STEP] key. You should be seeing the screen as depicted in Figure 9-35 below.

```
        EDIT/CREATE TIMELINE EVENT
Time({1.00})Min      Event(Hold For)
Input(Start)  Condition(off to on)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-35: The Edit/Create Timeline Event Screen with Hold For Event

j) In similar fashion create EDIT/CREATE TIMELINE EVENT screens for each of the other steps indicated in the Timeline Overview above. These are shown below. Do not forget to press [ADD STEP] after you have created each event screen so that these events will be added to your timeline. You should see [**SAVED**] where [ADD STEP] was before beginning to create the next event in your timeline. The following step closes contact CC1 and tells the recording device to start recording because an injection was made 0.6 seconds earlier.

```
        EDIT/CREATE TIMELINE EVENT
Time({1.01})Min      Event(Set Contact)
Set Contact(CC 1)    to (on)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-36: The Edit/Create Timeline Event Screen with Contact CC1 Closed

The following step opens contact CC1 so that the recording device does not immediately begin again after reaching its preset time to stop recording at 9.49 minutes.

```
        EDIT/CREATE TIMELINE EVENT
Time({1.03})Min      Event(Set Contact)
Set Contact(CC 1)    to (off)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-37: The Edit/Create Timeline Event Screen with Contact CC1 Opened

```
        EDIT/CREATE TIMELINE EVENT
Time({4.50})Min      Event(Range)
Channel(2)     Gain Range R(50nA)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

```
        EDIT/CREATE TIMELINE EVENT
Time({7.00})Min      Event(Range)
Channel(2)     Gain Range R(500nA)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-38: Additional Edit/Create Timeline Event Screens #1

k) The screens in Figure 9:38 show that the Coulochem III will change current range on channel 2 at 4.5 and 7.00 minutes which is 3.5 and 6.00 minutes after injection, respectively.

The screen below resets the current range to 10 μA before the next injection.

```
        EDIT/CREATE TIMELINE EVENT
Time({10.50})Min     Event(Range)
Channel(2)     Gain Range R(10uA)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-39: The Edit/Create Timeline Event Screen with a Current Range Change

```
        EDIT/CREATE TIMELINE EVENT
Time({10.51})Min     Event(Autozero)
This will produce an Auto Zero
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

```
        EDIT/CREATE TIMELINE EVENT
Time({10.52})Min     Event(Autozero)
This will produce an Auto Zero
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-40: Additional Edit/Create Timeline Event Screens #2

l) Again two autozero signals within one second will cause the *Coulochem III* to perform a full autozero over all current ranges except 1 mA. This will reduce baseline shifts as this program changes current ranges. 35 seconds are required for this type of autozero.

```
        EDIT/CREATE TIMELINE EVENT
Time({11.20})Min     Event(Loop)
Loop To(1.00)  Number of Loops(1000)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-41: The Edit/Create Timeline Event Screen with the Loop Command

m) The loop command will reset the timeline to 1.00 minutes and the program will await the next injection. One thousand loops have been entered so that the system will always be ready for the next injection. If you know in advance exactly how many injections will be made, then you should enter that number rather than 1000.

```
        EDIT/CREATE TIMELINE EVENT
Time({12.00})Min     Event(End)
This will Stop the timeline sequence
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 9-42: The Edit/Create Timeline Event Screen with the End Event

This will end the automatic current range changes and baseline rezeroing.

n) At this point you may wish to review your program. Move the cursor to the Time parameter and then use the ▲ or ▼ keys to scroll forward or backward through the timed event entries. When you are satisfied that your timeline program is correct, press the [NEXT] key to display the SAVE METHOD screen, Figure 9-31.

```
                SAVE METHOD
Save as method # (3) {()}
New method mode: Undefined Date:----
[CANCEL]            [PREVIOUS]  [SAVE]
```

Figure 9-43: The Save Method Screen

o) The cursor should be on the method number. Use the ▲ key to scroll to a method number that is unoccupied. In this case it's Method #3. Press the ENTER key to accept the Method number. The cursor will jump to another set of nested brackets on the same line. It is now possible to name your method.

p) To name your method CURRENT.RANGE.X:

Press the 9 key and then the ▲ key until a C is showing
Press the 0 key and then the ▼ key until a U is showing
Press the 0 key and then the ▼ key until an R is showing
Press the 0 key and then the ▼ key until an R is showing
Press the 9 key and then the ▼ key until an E is showing
Press the 0 key and then the ▼ key until an N is showing
Press the 0 key and then the ▼ key until a T is showing
Press the 0 key and then the ▼ key until a "." is showing
Press the 0 key and then the ▼ key until an R is showing
Press the 9 key and then the ▲ key until an A is showing
Press the 0 key and then the ▼ key until an N is showing
Press the 9 key and then the ▲ key until a G is showing
Press the 9 key and then the ▲ key until an E is showing
Press the 0 key and then the ▼ key until a "." is showing
Press the 0 key and then the ▼ key until an X is showing

By now your screen should look like Figure 9-44 below.

```
                SAVE METHOD
Save as method # (3){(CURRENT.RANGE.X)}
New method mode: Undefined Date:----
[CANCEL]            [PREVIOUS]  [SAVE]
```

Figure 9-44: The Save Method Screen with Added Method Name

Now press the ENTER key and then the [SAVE] key.

Messages about the saving process will be displayed and finally you will see the METHOD STORED screen as depicted here as Figure 9-45.

```
              METHOD STORED
Saved as method 3
Select the NEXT key to continue
                                  [NEXT]
```

Figure 9-45: The Method Stored Screen

The [NEXT] key will return you to the COULOCHEM MAIN MENU.

NOTE: Occasionally after saving a timeline step the [NEXT] key is pressed instead of the ENTER key. When this happens you will exit the Edit/Create Timeline Event screen and be asked to save your method. Simply press the [PREVIOUS] key to get back to where you were when you inadvertently pressed [NEXT].

Hopefully these last two examples have helped you understand the potential utility of the Timeline mode and how it is implemented.

10 Screen Mode Operation

10.1 Rationale for Screen Mode Operation

Ideally, the chromatographic separation provides a single electroactive compound to the detector at a given instant and the mobile phase does not contain any other electroactive species. In such a situation, the applied potential is set to the limiting potential for the peak of interest and a simple, straightforward measurement is obtained.

In some cases, chromatographic separation of the sample is quite difficult and the analyst cannot be certain that a given separation presents only a single electroactive compound to the detector at a given instant. When this occurs, the signal observed for the compound of interest is increased by the interferent and an erroneous result is obtained. This situation can frequently be solved by using a screen electrode immediately before the analytical electrode to eliminate possible electrochemical interferences.

A parallel problem is the possibility that the mobile phase contains trace levels of electroactive contaminants; this will increase the background current and thus reduce the limit of detection of the assay.

The size of these concerns is related to the potential needed to analyze the compound of interest; if a large potential is required, interferences may become significant.

This situation can frequently be alleviated by using a screen electrode immediately before the analytical electrode to eliminate possible electrochemical interferences. This is the basis for the two electrodes in ESA Model 501X Analytical Cells.

10.2 Using Screen Mode

If a screen electrode is used, its potential should be set so that co-eluting compounds and/or mobile phase contaminants are oxidized (or reduced) while the compound of interest is not affected. Typically, the potential of the first, or screen, electrode is set to the highest potential where no oxidation (reduction) of the analyte(s) occurs. This value is gleaned from the CV curve (see Section 13.3). This results in the electrolysis of all solutes in the mobile phase with formal potentials less than that of the analyte.

The screen electrode will eliminate all interferences if its potential corresponds to the limiting current wave of the interfering compounds. If the interferences are due to impurities in the mobile phase which oxidize (reduce) at a higher (lower) potential than the compound(s) of interest, an ESA Model 5020 Guard cell (Part Number 55-0417) can be placed immediately before the injector.

Typically, the potential of the Guard Cell will be set 50 mV higher than that of the working electrode of the analytical cell. Since the use of a two channel coulometric cell (e.g., a Model 501X cell) for the ESA Coulochem® III detector contains two electrodes, one electrode could be used as the screen electrode while the second electrode is used as the analytical electrode.

As an alternative, the first electrode could act as an analytical electrode at one potential with the second electrode acting as an analytical electrode at a higher potential. In this case, the first electrode acts as both a screen electrode and an analytical electrode. As an alternative, an ESA Model 5021 Conditioning Cell can be used before an analytical cell to function as a screen electrode.

To describe the use of the screen mode, we will consider the analysis of a two-component mixture, which has an overall series of HDVs as shown in Figure 10-1A. In this example, compounds A and B have similar retention times (the chromatogram using an absorbance detector is shown in Figure 10-1B).

- **Single Electrode Operation**

  If the potential to be used (E1) is applied to channel 1 (upstream electrode), compound A is easily quantitated. The observed chromatogram will appear as shown in Figure 10-1C. Compound B cannot be detected. If the potential to be used is E2 applied to channel 1, the resulting chromatogram will be as shown in Figure 10-1D. It will be difficult to quantitate either compound.

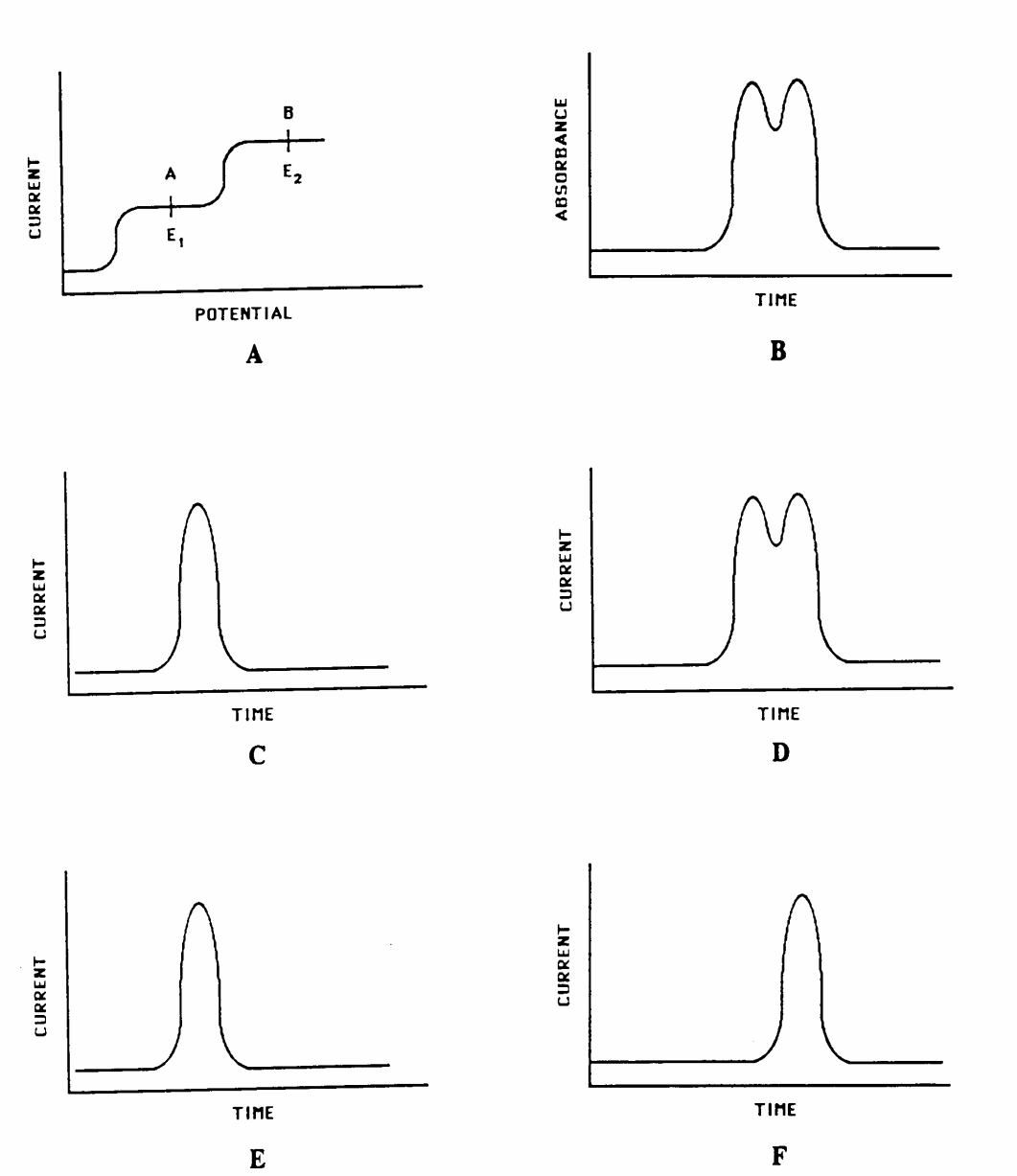
- **Two Electrode Operation**

  If the potential of the first electrode is set to E1 and the potential of the second electrode is set to E2, compound A will be detected by electrode 1 (Figure 10-1E) while compound B will be detected by electrode 2 (Figure 10-1F).

A two electrode cell is thus a very useful tool to improve selectivity in situations where efforts to increase the chromatographic resolution may be very difficult.

Screen mode operation can be summarized as follows:

- Impurities in the mobile phase and co-eluting species can often be electrolyzed at potentials less than that required for the compound of interest.
- The potential of the screen electrode should be chosen so that none of the analyte is electrolyzed (if very large detector potentials are used, it may be necessary to sacrifice some sensitivity by setting the screen potential on the rising portion of the wave of the compound of interest).
- The screen electrode must be coulometrically efficient.
- Electrolysis of interfering compounds must result in products, which will not react with the analyte at other electrodes.



A. Current/Voltage Curve for an Equimolar Mixture of Compounds A and B.
B. Chromatogram using an Absorbtion Detector.
C. Chromatogram using a Single Cell Electrochemical Detector Set at E1.
D. Chromatogram using a Single Cell Electrochemical Detector Set at E2.
E. Chromatogram using a Two Cell Electrochemical Detector. The first cell is set at E1 and the second cell is set at E2. Detector output is from E1.
F. Chromatogram using a Two Cell Electrochemical Detector. The first cell is set at E1 and the second cell is set at E2. Detector output is from E2.

Figure 10-1: Analysis of a Mixture Containing Two Electroactive Species that are not Well Resolved Chromatographically

It should be noted that although the screen mode of operation is a very powerful tool to improve selectivity, its use should not be considered as a solution for all interference problems. Cleanliness is a critical factor in any HPLC procedure; highly purified solvents should be used to minimize the concentration of possible electroactive species in the eluent. Additional efforts in the area of sample handling and sample clean up may be very useful to reduce interferences. In the same vein, improvements in the chromatographic resolution of closely spaced peaks should be attempted whenever possible.

11 Redox Mode Operation

11.1 Overview of Redox Mode Operation

If the analyte can undergo both an oxidation and a reduction process (e.g., some biogenic amines), the redox mode of electrochemical detection can be used to obtain very selective analyses. In the redox mode, the analyte is first oxidized, and then the oxidation product is reduced in a second step (or vice versa).

These processes are outlined in Equations 11-1 and 11-2.

$D^- \rightarrow D + e^-$	**(form the analytical species)**	**11-1**
$D + e^- \rightarrow D$	**(quantitation)**	**11-2**

In Redox mode, the upstream cell functions as an electrochemical "derivatizing agent" to form the species which is detected by the downstream cell.

The successful use of the redox mode of analysis depends on the following requirements:

a) The initial reaction must have a fixed stoichiometry (i.e., one mole of analyte must always generate the same amount of molecules of product).

b) The initial reaction must be reversible, with a high yield (ideally the yield should be 99.9% or better).

c) The initial reaction must be tolerant of small variations in chromatographic conditions and sample composition.

d) The applied potential for the initial oxidative or reductive process must correspond to the limiting current.

e) The electrolysis product should have a sufficiently long lifetime so that it can travel between the two electrodes.

f) The electrolysis product must be soluble in the mobile phase.

g) The analytes must be in the correct oxidation state.

In this discussion, we will consider the analysis of B by the redox mode via a reversible process. The current voltage curve, which describes the process, is shown in Figure 11-1. The upstream detector would be set at + 80 mV and converts B to A. The downstream detector is set at -40 mV and converts A back to B.

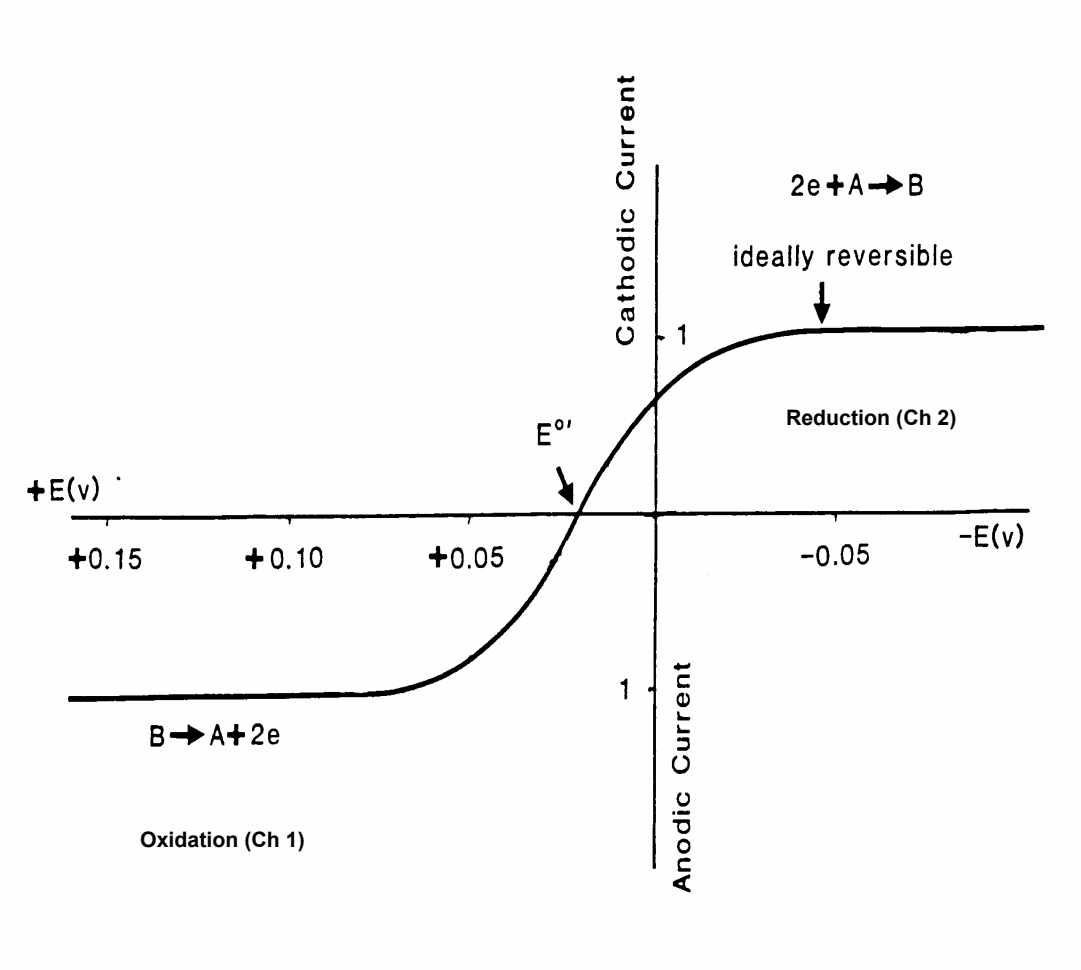


Figure 11-1: Current/Voltage Curve for an Ideally Reversible Couple using the Redox Mode

Some processes which are normally considered as irreversible by other electrochemical techniques such as DC polarography or cyclic voltammetry may be useful for redox mode analysis. In this situation, it is required that the two processes (conversion of A to B and the conversion of B to some other species) meet the general requirements described above.

The stability of the derivative is an important consideration. Typically, the distance between the reduction cell and the oxidation cell is such that the derivative must have a lifetime in the order of milliseconds to ensure that it can be detected by the second electrochemical cell.

11.2 Benefits of Redox Mode Operation

Redox mode can provide a significant degree of selectivity. In this discussion, we will assume that the sample contains an equimolar concentration of two electroactive species, A and B. The current-potential curves for A and B and the mixture C are presented in Figure 11-2. In this figure, E1 and E2 represent the oxidation potentials for A and B, while E4 and E5 represent the reduction potentials of A and B. To reduce the oxidation products from A and B respectively, E4 and E5 would be used.

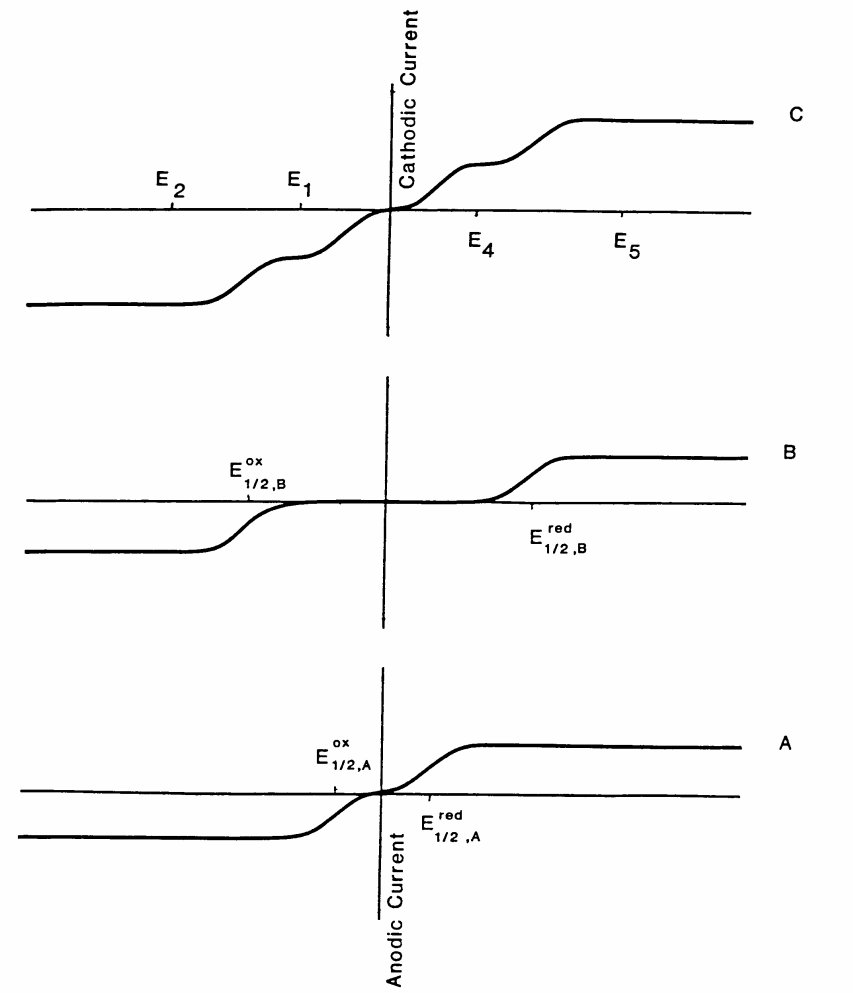


Figure 11-2: Current/Voltage Curve for a Mixture of Two Electroactive Compounds

By careful selection of the potentials, the specific compounds of interest can be detected. To determine which compound will be detected, the reader should use an analysis similar to that, which was used for screen mode.

11.3 Using Redox Mode

An additional benefit of the redox mode is the possible enhancement of sensitivity. For this discussion, we will consider the analysis of a compound that has a large oxidation half potential (e.g., 1200 mV). To detect the compound by direct oxidation, a potential at least 50-100 mV more positive than the half potential is required. The signal to noise ratio of such a measurement might be fairly poor if the mobile phase contains trace contaminants, which would be oxidized at 1300 mV. The cell lifetime would also be significantly reduced.

In the redox mode, the reduction of the oxidized product may be effected at a more reasonable potential than the oxidation of the original material. If this is the case, the presence of trace contaminants in the eluent may become less of an issue, and as a result the signal due to these contaminants will decrease. As the background noise decreases, the signal to noise (and thus the limit of detection) will increase.

In a redox mode detection procedure, there are three discrete stages:

- Initial electrolysis at electrode 1
- Mass transfer between the working electrodes
- Final electrolysis at electrode 2

The overall efficiency (N) of this process is defined by Equation 11-3:

$$N = \frac{(n2)(Q2)}{(n1)(Q1)} = \frac{(n2)(ip,2)}{(n1)(ip,1)} \qquad \textbf{11-3}$$

where: **n1** is the number of electrons in the first reaction
n2 is the number of electrons in the second reaction
Q1 is the peak area for electrode 1
Q2 is the peak area for electrode 2
ip,1 is the peak height for electrode 1
ip,2 is the peak height for electrode 2

If both electrodes are operating in a coulometrically efficient mode, the largest signal permitted by Faraday's law is observed and the generation of the electrochemical derivative is optimized (i.e., the collection efficiency is unity).

The collection efficiency may be less than unity if the reduced (oxidized) compound undergoes a reaction in the distance between the upstream and downstream electrodes. Such a process would lead to a decrease in the concentration of the electrochemical derivative that is observed at the second electrode.

A reaction that reduces the concentration of the newly formed electroactive species (B) could be due to its instability or due to a reaction of B with other materials, which may be present in the mobile phase (such as buffers). If decomposition does occur, it may still be possible to use the redox mode effectively, however, it will be necessary to carefully control the analytical conditions. To achieve a reproducible collection efficiency, conditions such as temperature, mobile phase ionic strength and pH, etc. should be carefully monitored.

An example of the utility of Redox Mode is presented in Figures 11-3 and 11-4.
In Figure 13-3, the chromatogram obtained when two electroactive compounds elute with a small differences in the retention time and oxidation potential is presented.

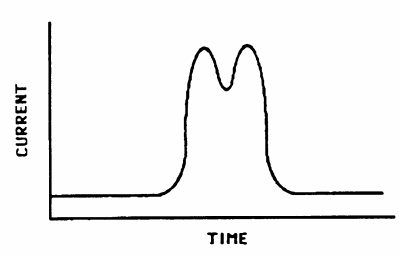


Figure 11-3: Co-elution of Electroactive Compounds

The potential of the first electrode is set so as to oxidize both compounds and the potential of the second electrode is set to only one of the compounds. The resulting much simpler chromatogram appears as shown in Figure 11-4. In this example redox mode has been used to favorably resolve a difficult chromatographic separation.

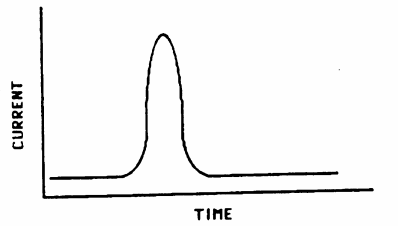


Figure 11-4: Detection of a Single Compound after an Oxidation Reaction

NOTE: The reduction trace will be inverted and a negative output voltage should be selected to present the chromatogram in the normal format (i.e., a peak appears as a positive deflection).

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12 Pulse Mode Operation

NOTE: Pulse Mode operation is provided via the optional *Pulse/Scan Potentiostat Board* (Pulse/Scan Upgrade Part Number 70-5504)

12.1 Overview of Pulse Amperometry

Accurate quantitation of an electroactive species via an electrochemical detector requires that the electrode surface be conditioned and the nature of the surface remains constant during the separation. Typically, the oxidation (reduction) product that is formed via the electrochemical process remains in solution and the condition of the electrode remains constant during the analysis. In some cases (e.g., the analysis of alcohols and carbohydrates) oxidation of the compounds of interest produces species that foul the electrode and will cause a decrease in the current as the electrochemical reaction proceeds.

NOTE: In this discussion, we are considering the situation where the performance of the electrode decreases rapidly during the electrochemical reaction. We note that over a period of time, all electrodes will eventually become fouled due to trace components in the sample and/or the mobile phase and over time may need to be cleaned as described in Chapter 5 (Coulometric Cells) and Chapter 6 (Amperometric Cells).

The technique of pulse amperometry allows detection of compounds which cannot be easily detected by a DC method. In DC Mode, the potential is held constant during the analysis. A typical three-pulse in the Pulse Mode operation includes the following steps (steps 2 and 3 are the "reconditioning steps"):

1) Measurement of the charge from the oxidation of the compound of interest.

2) The potential is set to a high positive potential for a short period of time.

3) The potential is set to a high negative potential for a short period of time.

4) The potential returns to that used for analysis.

NOTE: When pulse amperometric detection is employed, typically noble metal based electrodes such as gold or platinum are used. The use of a pulse waveform with a carbon or graphite electrode can cause irreversible damage to the electrode.

The pulse technique is described in Figure 12-1 for a three-pulse waveform. In this example (for the detection of a carbohydrate), the waveform is repeated every 700 msec, so that the detector can provide a data point to the recording device at that interval. Potential 1 is the analytical potential, while Potential 2 and Potential 3 are used to clean and reactivate the electrode, respectively. The duration of each of these steps can be selected by the analyst to obtain the optimum interval, since the interval size will affect the chromatographic resolution and sensitivity.

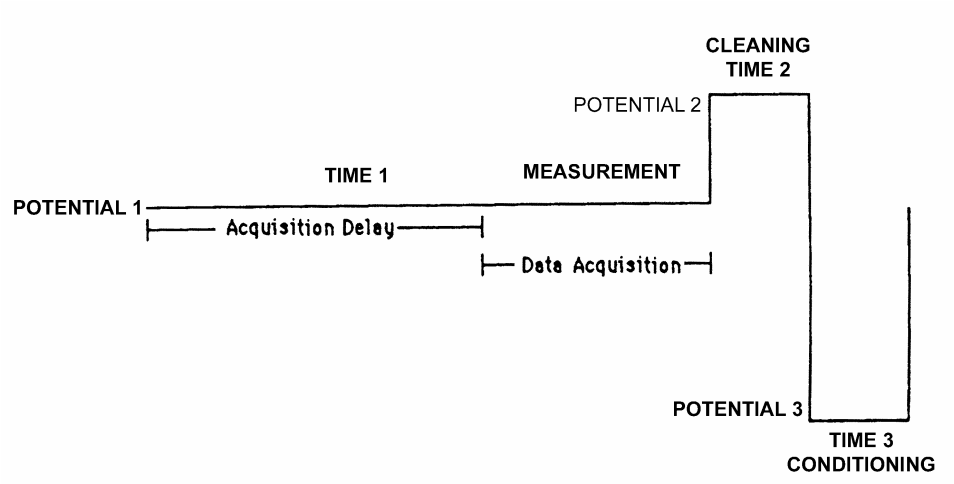

Figure 12-1: A Three Pulse Waveform used in the Pulse Mode of Electrochemical Detection

The purpose of the acquisition delay is to minimize the effect of the current that is caused when the analytical potential is first applied to the cell (due to the charging effects of the electrode acting as a capacitor). As the charging current falls off, the observed current is predominantly the current from the electrochemical process (see Figure 12-2). The second and third potentials (Potential 2 and Potential 3) simply serve to oxidize and reduce the electrode to present a renewed or activated electrode surface for the next measurement. (The *Coulochem III* detector can use either a three- or four-pulse waveform. Only the three-pulse waveform is discussed in this section.)

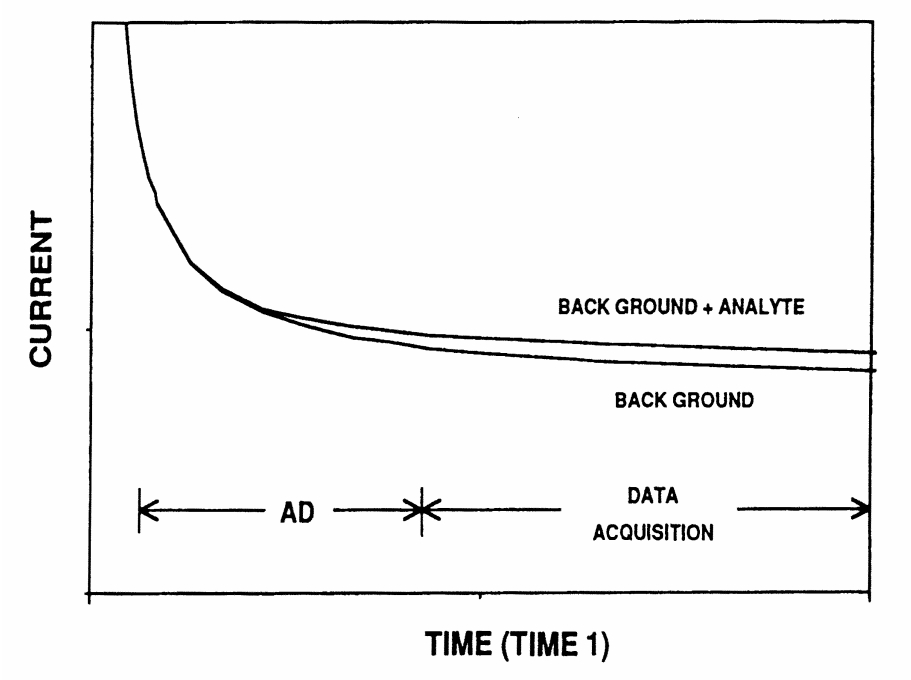

Figure 12-2: Decay of Electrode Current after Charging in the Presence and Absence of Analyte

During the current measurement period (data acquisition), the current is repeatedly sampled and integrated on a millisecond time interval. The integrated value (charge, in coulombs) is presented to the recording device once every waveform cycle.

To accurately quantitate the compound of interest, the detector integrates the current only during the measurement period. The *Coulochem® III* is capable of applying the three- or four- pulse waveforms and simultaneously presenting the desired signal.

Selecting the optimum conditions for pulse amperometry involves setting a number of parameters: the three or four potentials, the time for which each potential will be applied and the acquisition delay.

A timeline can be added to a pulse method in the same way that it can be added to a DC method. Programming and operation of a Pulse + Timeline method is described in Section 12.5 with sample pulse timeline methods in Sections 12.6 and 12.7.

12.2 Guidelines for Selecting Parameters in Pulse Mode

The following guidelines should be used when selecting parameters in Pulse Mode:

a) The analytical potential (Potential 1) should be set at the optimum potential for the compound of interest. (Literature values for a starting point and further optimized by performing an HDV.)

b) The conditioning potentials (Potential 2 and Potential 3) are usually set near the upper and lower limits for the mobile phase (i.e., the potentials at which the mobile phase begins to undergo an oxidative (reductive) reaction). These limits can be obtained by using the literature or by using cyclic voltammetry (Scan Mode), which is discussed in Chapter 13.

c) The selection of the data acquisition time is a compromise between two competing processes. A large value of Time 1 will provide a high value for the signal/noise (S/N) ratio; however, this reduces the number of data points in a given time interval and may lead to an insufficient number of data points to reproduce narrow peaks accurately.

d) The selection of the acquisition delay needs to be kept as short as possible to maximize the signal, but must be long enough to allow for most of the charging current, which would be seen as increased background signal and noise, to decay.

e) The conditioning times should be kept as short as possible to minimize the sampling time while maintaining appropriate reproducibility.

Pulse amperometry is commonly used to detect carbohydrates. While it should be recognized that the parameters will vary for each application, the following values provide an excellent starting point for the analysis of carbohydrates using the ESA Model 5040 cell and a four-pulse waveform. In order to maximize the sensitivity, these values (especially Potential 1) should always be optimized for the analysis of interest.

Potential 1 = + 100 mV	Time 1 = 500 msec
Potential 2 = - 1000 mV	Time 2 = 10 msec
Potential 3 = + 600 mV	Time 3 = 10 msec
Potential 4 = - 100 mV	Time 4 = 40 msec

Acquisition Delay (AD) = 300 msec

12.3 Generating a Pulse Method

12.3.1 Pulse Mode Parameters

The Pulse Mode Parameters are presented in Table 12-1.

Table 12-1: Pulse Mode Parameters

Parameter	Description	Range	Default
Filter	Smoothing function to reduce noise	None, Low, Medium, High	Medium
Run Time Security	Used to indicate if a password is needed to execute a run	Off, On User Selected Password	Off
Time 1	Time for acquisition delay and data acquisition	10 to 1000 msec (integer increments)	500 msec
Potential 1	Potential to be used for acquisition delay and data acquisition	-2000 mV to + 2000 mV (integer increments)	0 mV
Time 2	Time for elevated oxidation potential to condition electrode	2 to 1000 msec (integer increments)	100 msec
Potential 2	Potential to be used for elevated oxidation to condition electrode	-2000 mV to + 2000 mV (integer increments)	0 mV
Time 3	Time for reduction potential to condition electrode	0 to 1000 msec (integer increments)	100 msec
Potential 3	Potential to be used for reduction to condition electrode	-2000 mV to + 2000 mV (integer increments)	0 mV
Time 4	Time for reduction potential to condition electrode	0 to 1000 msec (integer increments)	0 msec
Potential 4	Potential to be used for reduction to condition electrode	-2000 mV to + 2000 mV (integer increments)	0 mV
Acq. Delay (Acquisition Delay)	Time before data is to be acquired	Time 2 msec to Time 1-5 msec (e.g., Time 1 is 500 msec, AD can be from 2 to 495 msec).	300 msec
Offset	Value added to each data point to offset chromatogram	-50 to + 50% (integer increments)	0 %
Range	Used to set the sensitivity level of the detector to ensure peaks are on scale	100 pC to 1 mC (step increments)	10 mC
Fill Scale Output	Indicates the voltage that is provided by the detector to drive the recording device	-1V, -0.1V, +0.1V, +1V	+ 1V

The time duration of an autozero in the Pulse Mode will vary widely with the timing parameters used and the amount of charge that must be zeroed out. Increasing the time of the pulse cycle (T1 + T2 + T3 + T4) will increase the time needed to complete an autozero.

Also, the amount of background charge on a specific range can significantly influence the duration of the autozero. For example, operating at a relatively low charge range with a background charge that is quite high - resulting in an over range situation - can result in a long autozero time. In this case the Coulochem III detector will display some or all of the following messages (Figure 12-3) to let you know what is occurring:

```
          Autozero Over Range
Setting maximum available autozero

                                [NEXT]
```

```
          PULSE METHOD x RUNNING
Autozero in progress
Compensating for over range
```

```
          PULSE METHOD x RUNNING
Autozero in progress
Compensation complete
```

Figure 12-3: Autozero Over Range Screens

These messages will be displayed for a short period of time before the standard Pulse Method Running screen is redisplayed. Usually, the detector will be able to autozero out the charge in an over range situation. However, if there is too much background charge for the selected range, the resulting signal will be off scale. In this case the range should be increased until the autozero brings the signal on scale.

12.3.2 Establishing/Editing a Pulse Mode Method

To establish/Edit a Pulse Mode Method:

a) Press [EDIT] on the COULOCHEM MAIN MENU to present the EDIT METHOD SELECTION Screen (Figure 12-4).

```
          EDIT METHOD SELECTION
Method Number (1) “”
Mode: (Pulse) Date:Jan 9, 2002 14:22
[CANCEL]                          [EDIT]
```

Figure 12-4: The Edit Method Selection Screen

b) If the Mode entry is not Pulse, use the ▲ or ▼ button to access Pulse mode, press [ENTER] and then press [Next] to present the PULSE MODE Screen #1 (Figure 12-5).

```
                PULSE EDIT
Filter (None)
Run Time Security is (Off)
[CANCEL]                          [NEXT]
```

Figure 12-5: The Pulse Edit Screen #1

c) Select the desired filter setting via the ▲ or ▼ button and press [ENTER]. The cursor will move to the Run Time Security field.

d) If run time security is desired, press the ▲ or ▼ button to present On. The cursor will move to the first character in the code field and you can enter a security code for this method, which can contain up to four digits. If the security code option is selected, the operator must enter the code to edit a parameter during a run. After you have entered the code, press [ENTER].

Press [NEXT] to access the PULSE EDIT Screen #2 (Figure 12-6).

```
                PULSE EDIT
Potential 1 {  0}mV Time 1{500}mS
Potential 2 {  0}mV Time 2{100}mS
[CANCEL]       [PREVIOUS]       [NEXT]
```

Figure 12-6: The Pulse Edit Screen #2

e) Edit the parameters on the PULSE EDIT Screen #2. A detailed discussion of each parameter, the range for each parameter and the mode of editing is presented in Table 12-1.

☞ NOTE: The general approach for entering values in PULSE mode is identical to that for DC Mode. As an example, if you want to edit Potential 1, move the cursor to that field and enter the desired value via the keypad. After you have entered the parameter, press [ENTER] to move the cursor.

f) After you have completed editing the PULSE EDIT screen #2, press [NEXT] to access edit PULSE EDIT screen #3 , which is identical to PULSE EDIT Screen #2, but presents fields for Potential 3/Time 3 and Potential 4/Time 4.

g) After you have completed editing PULSE EDIT Screen #3, press [NEXT] to access and edit PULSE EDIT Screen #4 (Figure 12-7).

```
                PULSE EDIT
Acq. Delay{300} mS    Offset(  0)%
Range R {100} mC  FS Output {1.0}V
[CANCEL]            [PREVIOUS]    [NEXT]
```

Figure 12-7: The Pulse Edit Screen #4

h) After you have completed editing the PULSE EDIT Screen #4, press [NEXT] to access the SAVE METHOD Screen (Figure 12-8), which is used to store the method in memory. When the screen is opened, the cursor will be placed on the method number.

```
               SAVE METHOD
Save as method # (2) {()}
New method mode: Undefined Date:----
[CANCEL]            [PREVIOUS]    [SAVE]
```

Figure 12-8: The Save Method Screen

The method number is the number assigned at the start of the editing of the method, but can be changed via the ▲ or ▼ buttons. After the method number has been selected, press [ENTER]. The cursor will move to the {()}field and you can enter additional information about the method (i.e., name of the method, your initials, etc. [e.g., 24]). The date and time will be automatically entered when you save the method.

Press [SAVE] to save the method. The method will be stored in memory and a prompt will be presented to press [NEXT] to continue.

12.4 Initiating and Performing a Pulse Mode Measurement

A Pulse Mode measurement operation can be initiated by opening a Pulse Method on the SELECT METHOD screen (Figure 12-9) and pressing [NEXT]. (To get to this menu from the Main Menu, press the [RUN] soft key.)

```
              SELECT METHOD
Method Number: (6) "DOGS"
Type:Pulse    Date:Jan. 3, 2002 13:44
[CANCEL]                          [RUN]
```

Figure 12-9: The Select Method Screen

When the [RUN] button is pressed on the Select Method screen, the method will be started and the PULSE METHOD 1 RUNNING Screen #1 (Figure 12-10) will be presented and the cells will be turned on.

```
          PULSE METHOD  1  RUNNING
E1{100}mV    T1{100}ms    AD{20}mS
R(100uC) 77.45nc 33%FS F{None} O(  0)%
[STOP]         [EDIT]      [PULSE 2-4]
```

Figure 12-10: The Pulse Method 1 Running Screen #1

In Figure 12-10 **E1** is Potential 1 or the analytical potential (mV)
T1 is the Time for Potential 1 (msec)
AD is the Acquisition Delay time (msec)
R is the full scale charge (coulombs)
The next field is the number of coulombs transferred (charge) or the signal output
F is the Filter selection
%FS is the Percent Full Scale for the present charge or signal out
O is the recorder or baseline Offset (%)

The buttons on the Pulse Method Running screen provide the following:

[STOP] halts the present method

[EDIT] places the cursor on E1 to edit the potential (parameters can be edited as described in Section 12.3).

[PULSE 2-4] accesses additional Pulse Mode parameters (Figure 12-11).

```
          PULSE METHOD  1  RUNNING
E2{100}mV      E3{100}mV     E4{100}mV
T2{100}mV      T3{100}mV     T4{100}mV
[STOP]   [EDIT]                [PULSE 1]
```

Figure 12-11: The Pulse Method 1 Running Screen #2

The potentials (E1, E2, E3 and E4), times (T1, T2, T3 and T4) and acquisition delay (AD) can be edited by moving the cursor to the appropriate field and using the keypad. The Range can be edited by moving the cursor to the appropriate field and change the value using the ▲ or ▼ button.

NOTE: Be sure that the CELL ON/OFF indicator light is ON, otherwise no potential will be applied to the electrodes.

NOTE: Changing the parameters during a run will not change the method on a permanent basis. If you want to save the changes, it will be necessary to edit the method as described in Section 12.3.

12.5 Pulse + Timeline Programming

The Pulse + Timeline mode feature allows the user to perform the following operations during a pulse data acquisition process:

- A potential can be placed on a cell or removed from a cell. In addition, you can change the potential.
- The range and filter settings can be changed.
- Autozero the detector.
- Open/close a contact closure.
- Place a marker on the detector output.
- Wait for a signal from an external device to perform an action such as autozeroing the detector.
- Autozero the detector.

In addition, Pulse + Timeline mode allows the use of looping operations, which are described in Section 12.5.3.

12.5.1 Establishing Pulse + Timeline Conditions

Pulse + Timeline mode programming involves setting the initial operating conditions as well as time based conditions. The initial conditions are set in the same manner as Pulse mode (which is described in detail in Section 12.3). For the convenience of the operator, we present the entire sequence of steps used to generate a Pulse + Timeline method in this section.

To set Pulse + Timeline mode operations:

a) Access the MODE SELECTION screen (Figure 12-12), by pressing EDIT on the main screen then EDIT on the EDIT METHOD SELECTION screen (after selecting the method number to be used). Then select PULSE+TL using the ▲ or ▼ buttons and then pressing [ENTER].

```
              MODE SELECTION
This method's Mode is DC
New Mode is (PULSE+TL)
[CANCEL]           [PREVIOUS]    [NEXT]
```

Figure 12-12: The Mode Selection Screen

b) Press [NEXT] to present the TIMELINE PULSE MODE Screen #1 (Figure 12-13).

```
           TIMELINE PULSE MODE
Filter (None)
Run Time Security is (Off)
[CANCEL]                          [NEXT]
```

Figure 12-13: The Timeline Pulse Mode Screen #1

c) Select the desired filter setting via the ▲ or ▼ button and press [ENTER]. The cursor will move to the Run Time Security field.

d) If run time security is desired, press the ▲ or ▼ button to present On. The cursor will move to the first character in the code field and you can enter a security code for this method, which can contain up to four digits. If the security code option is selected, the operator must enter the code to edit a parameter during a run. After you have entered the code, press [ENTER].

   Press [NEXT] to access the TIMELINE PULSE MODE Screen #2 (Figure 12-14).

```
           TIMELINE PULSE MODE
Potential 1 {  0}mV Time 1{500}mS
Potential 2 {  0}mV Time 2{100}mS
[CANCEL]          [PREVIOUS]     [NEXT]
```

Figure 12-14: The Timeline Pulse Mode Screen #2

e) Edit the parameters on the TIMELINE PULSE MODE Screen #2. A detailed discussion of each parameter, the range for each parameter and the mode of editing is presented in Table 12-1.

NOTE: The general approach for entering values in TIMELINE PULSE mode is identical to that for DC Mode. As an example, if you want to edit the Potential 1, move the cursor to that field and enter the desired value via the keypad. After you have entered the parameter, press [ENTER] to move the cursor.

f) After you have completed editing the TIMELINE PULSE MODE screen #2, press [NEXT] to access edit TIMELINE PULSE MODE screen #3, which is identical to PULSE EDIT screen #2 screen, but presents fields for Potential 3/Time 3 and Potential 4/Time 4.

g) After you have completed editing TIMELINE PULSE MODE screen #3, press [NEXT] to access and edit TIMELINE PULSE MODE screen #4 (Figure 12-15). A detailed discussion of each parameter, the range for each parameter and the mode of editing is presented in Table 12-1.

```
            TIMELINE PULSE MODE
Acq. Delay(300) mS    Offset(  0)%
Range R(100 µC) FS Output {1.0}V
[CANCEL]         [PREVIOUS]        [NEXT]
```

Figure 12-15: The Timeline Pulse Mode Screen #4

h) After you have completed editing the TIMELINE PULSE MODE Screen #4, press [NEXT] to edit the EDIT/CREATE TIMELINE EVENT Screen (Figure 12-16).

```
         EDIT/CREATE TIMELINE EVENT
Time ({0.00})         Event (Cell)
Cell(s) off
[     ]  [ADD STEP]   [PREVIOUS]   [NEXT]
```

Figure 12-16: The Edit/Create Timeline Event Screen

i) When the screen in Figure 12-16 is opened, the time when you want the event to occur can be edited. After you have indicated the time, press [ENTER] to access the Event field. The ▲ and ▼ buttons can be used to select the event that is to occur at the indicated time. As you select an event, the third line of the screen changes to present the option(s) for that event. A summary of the various events and how they are edited is presented in Table 12-2.

Table 12-2: Pulse Timeline Event Options

Event	Data Entry Options	Method of Editing	Comments
Cells	Off or On charge	▲ and ▼ buttons	This option is used to turn cells off after a run. See Note b.
Range	Pulse Charge Range	▲ and ▼ buttons	Range settable in steps: 10 pC-10 mC.
Set Potl	Pulse Number (1-4) Potential (E) in mV	▲ and ▼ buttons Numeric Keypad	The potential for each pulse can be set. See Note b.
Add Potl	Pulse Number (1-4) Potential increment to add in mV (negative potential values result in a decrease in potential)	▲ and ▼ buttons Numeric Keypad	The potential to be added to each pulse can be changed during a run. (Typically used with looping, see Note b and Section 12.5.3.)
Set Contact	Select Contact (CC1 to CC5) Select On or Off	▲ and ▼ buttons ▲ and ▼ buttons	External signal set on/off. Each contact closure can be set to on or off (closed or open) as desired.
Hold for	Input Condition (Cell Off, Autozero, Start) Condition Options are (off to on, on to off, off, on)	▲ and ▼ buttons ▲ and ▼ buttons	Indicate what the system should wait for to perform the action.
Autozero	This will produce an autozero		See Note a.
Marker	Marker Height (5) % FS	Numeric Keypad	Places an event marker on the output. Height can be set.
Reset	This will reset to initial pulse parameters		
Loop	Loop to (0.00) Number of Loops (0)	Numeric Keypad Numeric Keypad	Establish a set of repetitive operations. See Section 12.5.3 for details.
End	This will end the timeline sequence		
Pulse Time	Pulse Number (1-4) Pulse Time in msec	▲ and ▼ buttons Numeric Keypad	The time for each pulse can be set.

Note a: Typically an autozero operation takes place before an injection. If an autozero is performed during a run, it should be set at a point where the chromatogram is expected to be reasonably flat and the signal is low (i.e., the eluant does not contain electroactive compounds). For optimal performance, we suggest that a period of 15 sec be provided between an autozero and the onset of a peak.

Note b: It is recommended that an AUTOZERO be performed whenever a RANGE change or a CELL POTENTIAL change is performed. If a data station is used, set the data station to inhibit integration during the period from 0.1 minutes before and after the range change.

j) A timeline can include many events and each should be performed at a separate time. After you have edited a timeline step, press [ADD STEP] to present Figure 12-17, which confirms that the step has been saved. The [**SAVED**] label over the second soft key is presented to confirm that the step has been saved. When you enter a time for another step, the bottom line returns to that of Figure 12-16.

```
        EDIT/CREATE TIMELINE EVENT
Time(12.00) Min       Event (Cell)
Cell(s) (off)
[DELETE]  [**SAVED**]  [PREVIOUS]  [NEXT]
```

Figure 12-17: The Edit/Create Timeline Event [Saved**] Screen**

NOTE: The units of time used in the Pulse Mode + Timeline are in minutes and hundredths of minutes. (They are not in minutes and seconds.)

k) The display will then present a new event for editing and the [NEXT] button should be pressed after you have completed editing your timeline. To review/edit a previously entered step, press [PREVIOUS].

l) When you select [NEXT], the SAVE METHOD Screen (Figure 12-18) is used to store the method in memory. When the screen is opened, the cursor will be placed on the method number.

```
                SAVE METHOD
Save as method # (2) {()}
New method mode: Undefined Date:----
[CANCEL]         [PREVIOUS]      [SAVE]
```

Figure 12-18: The Save Method Screen

m) Select the desired method number and press [ENTER]. The {()} field will be activated and you can add a name to the method. Press a numeric button to enter a character, the press the ▲ or ▼ buttons to select the desired character. After the number 9 is presented, alphabetic characters are presented, so that you can enter names such as ABC. The *New Method Mode* and the *Date* fields will be automatically filled in when the method is saved.

n) When you press SAVE, the method will be saved and a screen will be presented indicating that you should press the [NEXT] button to continue. This will present the COULOCHEM MAIN MENU.

12.5.2 Performing a Pulse + Timeline Operation

To Run a Pulse + Timeline Method:

a) Press RUN on the COULOCHEM MAIN MENU to present the SELECT METHOD Screen (Figure 12-19).

```
                SELECT METHOD
Method Number: (1)"ABC"
Type:Pulse+TL    Date:Jan 5,2002 8:22
[CANCEL]                          [RUN]
```

Figure 12-19: The Select Method Screen

b) Use the ▲ or ▼ buttons to select the desired method. When the desired method is presented, press [RUN] to present the PULSE + TL METHOD RUNNING screen (Figure 12-20). This is the first of four screens that describe the operation of the PULSE method (Figure 12-21 and Figure 12-24). The parameters indicated on this screen are described in Table 12-1, and can be edited as described below.

```
PULSE TL METHOD 2   RUNNING   TIME=0.00
E1{500}mV T1{100} mS AD{ 20} mS
R{  10mC} 333uC  0%FS: F(None) O(0)%
[STOP]  [EDIT]    [EVENTS]   [PULSE 2-4]
```

Figure 12-20: The Pulse TL Method 2 Running Screen #1

c) The [PULSE 2-4] button presents Figure 12-21, which includes the values for E2-E4 and T2-T4.

```
PULSE TL METHOD 2   RUNNING   TIME=0.00
E2{500}mV    E3{100} mV     E4{333}mV
T2{100)mS    T3{100}mS      T4{100}mS
[STOP]  [EDIT]    [EVENTS] [PULSE 1]
```

Figure 12-21: The Pulse TL Method 2 Running Screen #2

The EVENTS button presents the EVENTS screen (for an example, see Figure 12-22), which describes the various events in the timeline. The [PULSE 1] and [PULSE 2-4] buttons access Figure 12-19 and Figure 12-20, respectively. If there is more than one event in the timeline, the additional events can be accessed via the ▲ or ▼ button.

```
PULSE TL METHOD 2   RUNNING   TIME=0.00
Time(0.44)Min.       Event: Range
Range: 10 mC
[STOP]         [PULSE 1]    [PULSE 2-4]
```

Figure 12-22: The Pulse TL Method 2 Running Screen #3

12.5.3 Establishing a Loop

A loop is employed when a step or series of steps is to be performed on a repetitive basis during the experiment. A common application of a loop is to quantitate a sample at a variety of potentials by taking a measurement, then incrementing the potential by 50 mV. This process can be automated by the LOOP command and the ADD POTL command.

To establish a loop:

a) Access the EDIT/CREATE TIMELINE EVENT Screen (Figure 12-23).

```
        EDIT/CREATE TIMELINE EVENT
Time(0.00) Min.       Event (Cell)
Cell(s) (off)
[    ]   [ADD STEP]   [PREVIOUS]   [NEXT]
```

Figure 12-23: The Edit/Create Timeline Event [Cell] Screen

b) Select the desired time at which the loop should be started (e.g., 7.00 min) and press [ENTER].

c) Use the ▲ or ▼ buttons to access LOOP in the EVENT field (Figure 12-24). The Loop to field is used to enter the program time that you want to go back to (e.g., 3.50 min). Enter in the Loop To time.

```
        EDIT/CREATE TIMELINE EVENT
Time(0.00) Min       Event (Loop)
Loop To {0.00}   Number of Loops {0}
[    ]   [ADD STEP]   [PREVIOUS]   [NEXT]
```

Figure 12-24: The Edit/Create Timeline Event [Loop] Screen

d) Enter the number of loops that you want to perform.

e) Press [ADD STEP] to store the step and present a new EDIT/CREATE TIMELINE EVENT Screen.

f) Enter the desired time, event (e.g., Add Potl) and potential to add. The time for this event should be greater than the time to which you are looping to. An example of this order is:

   i) Set the initial potential to 500 mV.

   ii) At 7.00 minutes, set a loop event and loop to 4.00 minutes and set the number of times the loop should be repeated (Number of Loops).

   iii) At 4.25 minutes, set an Add Potl event and program potential to add 25 mV. Select Pulse Number 1.

   If this example is used:

   i) For the first 7.00 minutes the potential of pulse 1 will be 500 mV.

   ii) At 7.00 minutes, the clock will return to 4.00 minutes.

   iii) At 4.25 minutes, the potential of pulse 1 will be set to 525 mV.

   iv) At 7.00 minutes, the loop will be reset and the clock will again be set to 4.00 minutes.

   v) At 4.25 minutes, the potential of pulse 1 will be set to 550 mV.

   Steps (ii)-(v) will be performed for the number of loops indicated as in Figure 12-24. After the last loop is completed, the system will continue at the last potential until the STOP button is pressed.

12.6 Using Timeline to Generate Charge Voltage Curves or Hydrodynamic Voltammograms for Pulse

Successful operation of an electrochemical detector requires advanced knowledge of the optimum applied potential(s). This information is called a Current Voltage (CV) Curve or a Hydrodynamic Voltammogram (HDV) and one is usually generated for each analyte of interest. HPLC makes this task much simpler because mixtures of all the analytes of interest and their major impurities are separated before flowing through the EC detector. Since the generation of a HDV is such an important part of method development we will create a timeline for Pulse Mode that performs that task as an example of the utility of timeline.

The two key timeline commands used in this effort are called Loop and Add Potl. Assume that your HPLC system consists of an autosampler, an integrator, isocratic pump, column, and a Coulochem III Electrochemical Detector with the installed Pulse/Scan option. Assume also that all analytes and other peaks have eluted by nine minutes after injection and that you wish to generate a HDV from +350 mV on the electrode in your cell to +100 mV. This potential will be varied only for Pulse 1 (the analytical potential) in an application for a four pulse method.

NOTE: In general, once a given pulse method has been established (either from the literature or your own method development) only the potential for Pulse 1 need be optimized by determination of a HDV.

Connections

1. Connect the CC1 output contact closure on the Coulochem III to the input trigger on the autosampler such that closure of the contact will trigger an injection.

2. Connect the autosampler output contact closure to the recording device input (integrator or data station) such that the recording device will begin recording when the autosampler signals that an injection has been completed. Set the recording device's input voltage to +1 volt and the recording time to 9.00 minutes.

Coulochem III Pulse Timeline Program Overview

Time	Event	Device Identity	Device State	Comments
0.00	Set Contact	CC1	On	Causes Injection
0.02	Set Contact	CC1	Off	Resets CC1
10.00	Add Potl	Pulse 1	-50 mV	Increments Pulse 1 Potl
12.00	Autozero	Rezeros baseline after potl change		
12.50	Loop	Loop to 0.00 min	# of Loops = 5	Back to Inject
13.00	Set Potl	Pulse 1	+350 mV	Resets Pulse 1 Potl
14.00	End	This will stop the timeline sequence		

Creating this Method

a) Access the EDIT MODE SELECTION Screen (Figure 12-25), by pressing EDIT on the COULOCHEM MAIN MENU.

```
          EDIT MODE SELECTION
Method Number: (1) ""
Mode: DC   Date: January 26,2002 13:57
[CANCEL]                        [EDIT]
```

Figure 12-25: The Edit Mode Selection Screen

b) Press [EDIT] to access the MODE SELECTION Screen (Figure 12-26). The cursor will be in the New Mode field.

```
             MODE SELECTION
This method's Mode is DC
New Mode is (DC) with (2) Channel(s)
[CANCEL]            [PREVIOUS]  [NEXT]
```

Figure 12-26: The Mode Selection Screen

c) Move the ▲ or ▼key until PULSE+TL is indicated and press [ENTER]. Press the [NEXT] key to see the Filter and Run Time Security Screen, Figure 12-27.

```
          TIMELINE PULSE MODE
Filter (None)
Run time security is (off)
[CANCEL]                        [NEXT]
```

Figure 12-27: The Timeline Pulse Mode Screen #1

Assume we do not wish to use a filter or to run in secure mode.

d) Press the [NEXT] key to access the TIMELINE PULSE MODE Screen #2 (Figure 12-28).

```
          TIMELINE PULSE MODE
Potential 1{0}mV   Time 1{500}mS
Potential 2{0}mV   Time 2{100}mS
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 12-28: The Timeline Pulse Mode Screen #2

e) Screen #2 is used to set pulse potential 1 and 2 as well as their time durations. When the screen is accessed, the cursor will appear on the first character of the *Potential 1* field. Enter 350 mV using the numeric keypad. After the potential has been set, press [ENTER] to move the cursor to the *Time 1* field. Select 500 mS via the numeric keypad (500 mS is the default value). After Time 1 has been set, press [ENTER] to move the cursor to the *Potential 2* field. Enter in a potential of 2000 mV using the numerical keypad. Then press [ENTER]. Now the cursor will be at the *Time 2* field. Enter in a time of 10 mS using the numerical keypad. After these parameters have been set, press [NEXT] to present an additional screen of parameters for TIMELINE PULSE (Figure 12-29). The cursor will appear on the *Potential 3* field.

```
            TIMELINE PULSE MODE
Potential 3{0}mV   Time 3{100}mS
Potential 4{0}mV   Time 4{0}mS
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 12-29: The Timeline Pulse Mode Screen #3

f) As above, set the potentials and times for pulses 3 and 4. Enter in the following values: Potential 3 = 600 mV, Time 3 = 10 mS, Potential 4 = -100 mV and Time 4 = 40 mS. After these parameters have been entered press [NEXT] to go to the next screen (Figure 12-30).

```
            TIMELINE PULSE MODE
Acq. Delay { 300}mS     Offset (  0)%
Charge Range R{100 µC}   FS Output {1.0}V
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 12-30: The Timeline Pulse Mode Screen #4

g) Enter in 300 mS (the default value) for the Acq. Delay, 0 for the baseline Offset, 10 µC for the Charge Range and a FS Output of 1.0 V.

h) After you have completed the editing of the above screen, press [NEXT] to present the EDIT/CREATE TIMELINE EVENT Screen (Figure 12-31), which is used to schedule events in the timeline.

```
        EDIT/CREATE TIMELINE EVENT
Time({0.00}) Min.     Event (Cell)
Cell(s) (off)
[     ]  [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 12-31: The Edit/Create Timeline Event Screen

i) Press [ENTER] to accept the first time as (0.00) Min and to access the Event field. Use the ▲ key to display Set Contact. Press [ENTER] again to accept that the Event at 0.00 minutes will be to set a contact closure. Press [ENTER] again to accept that you wish to set CC1 (which is connected to the autosampler inject input). Use the ▲ key to set the state of CC1 to On (or Closed).

At this point you have created the first line in your timeline program. (See the Timeline Program Overview above). **HOWEVER you have not saved it.**

j) You must remember to press the [ADD STEP] key after every step is created. Press [ADD STEP]. You will see the Saved Screen, Figure 12-32.

```
        EDIT/CREATE TIMELINE EVENT
Time({0.00}) Min.    Event(Set Contact)
Set Contact(CC1)     to (on)
[DELETE]  [**SAVED**]  [PREVIOUS] [NEXT]
```

Figure 12-32: The Edit/Create Timeline Event [Saved**] Screen**

k) When the autosampler has finished its injection sequence it will again check the status of its inject command contact closure. By that time we must have opened (turned it off) the CC1 contact or another injection will occur. So our second timeline step must be to turn CC1 off again.

Press [ENTER] to see the following (Figure 12-33):

```
        EDIT/CREATE TIMELINE EVENT
Time({0.00}) Min.    Event (Set Contact)
Set Contact(CC1)     to (on)
[     ]  [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 12-33: The Edit/Create Timeline Event Screen with Set Contact Event Already Presented

l) The cursor will be on the Time parameter. Press the 0 key twice and then press the 2 key. Press [ENTER] three times to reach the on/toggle/off parameter. Use the ▼ key to display off. You should now see:

```
        EDIT/CREATE TIMELINE EVENT
Time({0.02}) Min.    Event (Set Contact)
Set Contact(CC1)     to (off)
[      ] [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 12-34: The Edit/Create Timeline Event Screen with Set Contact CC1 Turned OFF at 0.02 Minutes

m) **Press [ADD STEP] to save**, and then press [ENTER] to return to the Time Parameter.

n) In like manner create the **Saved** Timeline Event Screens shown below in Figure 12-35.

```
        EDIT/CREATE TIMELINE EVENT
Time({10.00}) Min.   Event(Add Potl)
Pulse number(1)      Add Potl(-50)mV
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

```
        EDIT/CREATE TIMELINE EVENT
Time({12.00}) Min.   Event(Autozero)
This will produce an Autozero
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

```
        EDIT/CREATE TIMELINE EVENT
Time({12.50}) Min.   Event(Loop)
Loop To (0.00) Number of Loops(5)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-35: The Edit/Create Timeline Event [Saved**] Screens**

As each loop occurs the Pulse 1 potential will change from 350 mV to 300 mV to 250 mV to 200 mV to 150 mV to 100 mV. An injection will occur and a chromatogram will be recorded at each Pulse 1 potential. We allow two minutes for the baseline to stabilize after a potential change before autozeroing.

```
        EDIT/CREATE TIMELINE EVENT
Time({13.00}) Min.   Event(Set Potl)
Channel(2)     Potential E(350)mV
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-36: The Edit/Create Timeline Event Screen #1

Finally we reset the Pulse 1 potential back to its original value of 350 mV before stopping the experiment at 14.00 minutes. Please add the step at 13.00 minutes (shown above in Figure 12-36) and the final step at 14.00 minutes (shown below in Figure 12-37) to the timeline program you are creating.

```
        EDIT/CREATE TIMELINE EVENT
Time({14.00}) Min.   Event(End)
This will Stop the timeline sequence
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-37: The Edit/Create Timeline Event Screen #2

o) Before saving this method you should review the Timed Events portion. Press the [ENTER] key until the cursor is on the Time parameter. Now use the ▲ and ▼ keys to step through the times saved in the Timeline. As you step to each time the event programmed for that time will be displayed. When you are satisfied that your timeline program is correct, press the [NEXT] key to display the SAVE METHOD screen, Figure 12-38.

```
                SAVE METHOD
Save as method # (2) {()}
New method mode : Undefined Date:----
[CANCEL]             [PREVIOUS]  [SAVE]
```

Figure 12-38: The Save Method Screen

p) The cursor should be on the method number. Use the ▲ and ▼ keys to scroll to a method number that is unoccupied. In this case it is Method #2. Press the [ENTER] key to accept the Method number. The cursor will jump to another set of nested brackets on the same line. It is now possible to name your method.

q) To name your method CV.CURVE:

Press the 9 key and then the ▲ key until a C is showing
Press the 0 key and then the ▼ key until a V is showing
Press the 0 key and then the ▼ key until a "." is showing
Press the 9 key and then the ▲ key until a C is showing
Press the 0 key and then the ▼ key until a U is showing
Press the 0 key and then the ▼ key until an R is showing
Press the 0 key and then the ▼ key until a V is showing
Press the 9 key and then the ▲ key until a E is showing
Now press the [ENTER] key and then the [SAVE] key

Messages about the saving process will be displayed and finally you will see the METHOD STORED Screen as depicted here as Figure 12-39.

```
               METHOD STORED
Saved as method 2.
Select the NEXT key to continue
                                  [NEXT]
```

Figure 12-39: The Method Stored Screen

The [NEXT] key will return you to the COULOCHEM MAIN MENU.

r) Had we wished we could have also turned the cells off to protect them until we returned to check our results. Some pumps have a STOP FLOW contact closure. We could have wired CC2 to this and stopped mobile phase flow after turning the cell off before we ended the method. There are other options as well. Some of these will be explored in the next example, Changing Charge Ranges.

☞ NOTE: Occasionally after saving a timeline step the [NEXT] key is pressed instead of the [ENTER] key. When this happens you will exit the Edit/Create Timeline Event screen and be asked to save your method. Simply press the [PREVIOUS] key to get back to where you were when you inadvertently pressed [NEXT].

12.7 Using Timeline to Change Charge Ranges Reproducibly During Each Analytical Chromatographic Run

In this second example assume that your chromatographic system is very simple. It consists of a pump, a manual injector with position sensing switch, a column, a Coulochem III detector, and a simple chromatographic recording peak integrator. Also assume that your samples are quite complex yet you have perfected a separation that isolates the peaks that you need to quantitate. The only problem is that the concentration of the analytes varies widely so you need to somehow change detector sensitivity in a reproducible fashion at various times during each chromatographic run in order to keep large peaks on scale and small peaks are enlarged for better quantitation.

Changing detector sensitivity at various times during development of each chromatogram requires precise timing and a knowledge of when sample injection has occurred. Assume that you have run a few chromatograms manually and now know that Peaks A, B, and C elute at 2.5, 4.5, and 6.5 minutes, respectively. You also know that probable concentration ranges will be such that 10 μC, 50 nC, and 500 nC detector charge ranges are optimum for peaks A, B, and C, respectively and that a pulse 1 potential of +200 mV is all that is required to oxidize all three compounds. (Also, assume that the standard four-pulse method listed above is acceptable.)

Connections

1. Connect the CC1 output contact closure of the Coulochem III to the input trigger on the recording integrator such that closure of the contact will trigger the start of data recording. Set the recording integrator input range to 1 volt and recording time to 9.49 minutes.

2. Connect the Coulochem III PULSE/SCAN SIGNAL OUT BNC connector to the "Data In" terminals (or equivalent) on the recording integrator.

3. Connect the injector position sensing leads to the START input contact closure of the Coulochem III so that every injection will trigger an automatic timed series of events.

Coulochem III Timeline Program Overview

Time	Event	Device Identity	Device State	Comments
0.20	Autozero			
1.00	Hold For	Start	OFF to ON	Waits for sample injection.
1.01	Set Contact	CC1	ON	Starts Data Integrator
1.03	Set Contact	CC1	OFF	Resets CC1
4.50	Range		50 nC	Ready for Peak B
5.52	Autozero			
7.00	Range		500 nC	Ready for Peak C
7.02	Autozero			
10.50	Range		10 μC	Ready for Peak A
10.52	Autozero			
11.60	Loop	Loop to 1.00 min	# of Loops = 1000	Back to Inject
121.00	End	This will stop the timeline sequence		

NOTE: Unlike for DC Mode, there is no All Range Autozero for the Pulse Mode. Autozero will only reset the baseline to zero for the range that is currently active. An additional autozero should be performed after each range change.

Creating this Method

a) Access the MODE SELECTION screen by pressing EDIT on the COULOCHEM MAIN MENU. And use the ▲ key to scroll up to an unused method as shown in Figure 12-40.

```
          EDIT METHOD SELECTION
Method Number: (3) ""
Mode: Undefined Date: -,-,- -:
[CANCEL]                        [EDIT]
```

Figure 12-40: The Edit Method Selection Screen

b) Press [EDIT] to access the MODE SELECTION Screen (Figure 12-41). The cursor will be in the New Mode field.

```
                MODE SELECTION
This method's Mode is DC
New Mode is (DC) with (2) Channel(s)
[CANCEL]              [PREVIOUS]  [NEXT]
```

Figure 12-41: The Mode Selection Screen

c) Press the ▲ or ▼keys until Pulse+TL is indicated and press [ENTER]. Press the [NEXT] key to see the TIMELINE PULSE MODE Filter and Run Time Security Screen, Figure 12-42.

```
            TIMELINE PULSE MODE
Filter (None)
Run time security is (off)
[CANCEL]                          [NEXT]
```

Figure 12-42: The Timeline Pulse Mode Screen #1

Assume we do not wish to use a filter or run in secure mode.

d) Press the [NEXT] key to see the TIMELINE PULSE MODE Screen #2 (Figure 12-43).

```
            TIMELINE PULSE MODE
Potential 1{0}mV   Time 1{500}mS
Potential 2{0}mV   Time 2{100}mS
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 12-43: The Timeline Pulse Mode Screen #2

e) Screen #2 is used to set pulse potential 1 and 2 as well as their time durations. When the screen is accessed, the cursor will appear on the first character of the Potential 1 field. Enter 200 mV using the numeric keypad. After the potential has been set, press [ENTER] to move the cursor to the Time 1 field. Select 500 mS via the numeric keypad (500 mS is the default value). After Time 1 has been set, press [ENTER] to move the cursor to the Potential 2 field. Enter in a potential of 2000 mV using the numerical keypad. Then press [ENTER]. Now the cursor will be at the Time 2 field. Enter in a time of 10 mS using the numerical keypad. After these parameters have been set, press [NEXT] to present an additional screen of parameters for TIMELINE PULSE (Figure 12-44). The cursor will appear on the Potential 3 field.

```
            TIMELINE PULSE MODE
Potential 3{0}mV   Time 3{100}mS
Potential 4{0}mV   Time 4{0}mS
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 12-44: The Timeline Pulse Mode Screen #3

f) As above, set the potentials and times for pulses 3 and 4. Enter in the following values: Potential 3 = 600 mV, Time 3 = 10 mS, Potential 4 = -100 mV and Time 4 = 40 mS. After these parameters have been entered press [NEXT] to go to the next screen (Figure 12-45).

```
            TIMELINE PULSE MODE
Acq. Delay { 300}mS     Offset (  0)%
Charge Range R{100 µC}   FS Output {1.0}V
[CANCEL]             [PREVIOUS]  [NEXT]
```

Figure 12-45: The Timeline Pulse Mode Screen #4

g) Enter in 300 mS (the default value) for the Acq. Delay, 0 for the baseline Offset, 10 µC for the Charge Range and a FS Output of 1.0 V.

h) After you have completed the editing of the above screen, press [NEXT] to present the EDIT/CREATE TIMELINE EVENT Screen #1 (Figure 12-46), which is used to schedule events in the timeline.

```
        EDIT/CREATE TIMELINE EVENT
Time({0.00}) Min.     Event (Cell)
Cell(s) (off)
[     ]  [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 12-46: The Edit/Create Timeline Event Screen #1

i) Press 20 to enter the first time as (0.20) min. Press [ENTER] to access the Event field. Use the ▲ key to display Autozero. Press [ENTER] again to accept Autozero at 0.20 minutes. This will move the cursor to the Input choice. Press [ENTER] again. The EDIT/CREATE TIMELINE EVENT screen should now look like Figure 12-47 below. Press [ADD STEP] to save this first step in the timeline.

```
        EDIT/CREATE TIMELINE EVENT
Time({0.20})Min.     Event(Autozero)
This will produce an Autozero
[     ]  [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 12-47: The Edit/Create Timeline Event Screen with Autozero Event

Now create a situation where the timeline will pause until an injection is made. For simplicity change the time to 1.00 minutes by pressing the 1 key and then the 0 key twice. Press [ENTER] to move the cursor to the event field and then press the ▼ key to display the Hold For event. Again press [ENTER] to reach the input field and press the ▼ key to display Start. Press [ENTER] to accept Start and access the condition field.

Press the ▼ key until the condition off to on is displayed. Press [ENTER] and then press the [ADD STEP] key. You should be seeing the screen as depicted in Figure 12-48 below.

```
        EDIT/CREATE TIMELINE EVENT
Time({1.00})Min.     Event(Hold For)
Input(Start)  Condition(off to on)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-48: The Edit/Create Timeline Event Screen with Hold For Event

j) In similar fashion create EDIT/CREATE TIMELINE EVENT screens for each of the other steps indicated in the Timeline Overview above. These are shown below. Do not forget to press [ADD STEP] after you have created each event screen so that these events will be added to your timeline. You should see [**SAVED**] where [ADD STEP] was before beginning to create the next event in your timeline. The following step closes contact CC1 and tells the recording device to start recording because an injection was made 0.01 minutes earlier.

```
        EDIT/CREATE TIMELINE EVENT
Time({1.01})Min.     Event(Set Contact)
Set Contact(CC1)     to (on)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-49: The Edit/Create Timeline Event Screen with Set Contact CC1 Closed Event

The following step opens contact CC1 so that the recording device does not immediately begin again after reaching its preset time to stop recording at 9.49 minutes.

```
        EDIT/CREATE TIMELINE EVENT
Time({1.03})Min.     Event(Set Contact)
Set Contact(CC 1)    to (off)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-50: The Edit/Create Timeline Event Screen with Set Contact CC1 Opened Event

```
        EDIT/CREATE TIMELINE EVENT
Time({4.50})Min.     Event(Range)
Pulse Charge Range R(50nC)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

```
        EDIT/CREATE TIMELINE EVENT
Time({4.52})Min.     Event(Autozero)
This will produce an Autozero
[     ]  [ADD STEP]  [PREVIOUS]  [NEXT]
```

```
        EDIT/CREATE TIMELINE EVENT
Time({7.00})Min.     Event(Range)
Pulse Charge Range R(500nC)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

```
        EDIT/CREATE TIMELINE EVENT
Time({7.02})Min.     Event(Autozero)
This will produce an Autozero
[     ]  [ADD STEP]  [PREVIOUS]  [NEXT]
```

Figure 12-51: Additional Edit/Create Timeline Event Screens #2

k) The screens in Figure 12-51 show that the Coulochem III will change range at 4.50 and 7.00 minutes, which is 3.50, and 6.00 minutes after injection, respectively. There will also be an autozero 0.02 minutes after each range change.

Figure 12-52 below resets the charge range to 10 μC before the next injection.

```
        EDIT/CREATE TIMELINE EVENT
Time({10.50})Min.    Event(Range)
Pulse Charge Range R(10uC)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-52: The Edit/Create Timeline Event Screen with Range Change Event

```
        EDIT/CREATE TIMELINE EVENT
Time({10.52})Min.    Event(Autozero)
This will produce an Autozero
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-53: Additional Edit/Create Timeline Event Screen #3

l) Again an autozero is performed after the range change (Figure 12-53).

```
        EDIT/CREATE TIMELINE EVENT
Time({11.20})Min.    Event(Loop)
Loop To(1.00)  Number of Loops(1000)
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-54: The Edit/Create Timeline Event Screen with Loop Command Event

m) The loop command (Figure 12-54) will reset the timeline to 1.00 minutes and the program will await the next injection. One thousand loops have been entered so that the system will always be ready for the next injection. If you know in advance exactly how many injections will be made, then you should enter that number rather than 1000.

```
        EDIT/CREATE TIMELINE EVENT
Time({12.00})Min.    Event(End)
This will Stop the timeline sequence
[DELETE] [**SAVED**] [PREVIOUS]  [NEXT]
```

Figure 12-55: The Edit/Create Timeline Event Screen with End Event

This (Figure 12-55) will end the automatic charge range changes and baseline rezeroing.

n) At this point you may wish to review your program. Move the cursor to the Time parameter and then use the ▲ or ▼ keys to scroll forward or backward through the timed event entries. When you are satisfied that your timeline program is correct, press the [NEXT] key to display the SAVE METHOD Screen, Figure 12-56.

```
                SAVE METHOD
Save as method # (3) {()}
New method mode: Undefined Date:----
[CANCEL]            [PREVIOUS]  [SAVE]
```

Figure 12-56: The Save Method Screen

o) The cursor should be on the method number. Use the ▲ key to scroll to a method number that is unoccupied. In this case it's Method #3. Press the [ENTER] key to accept the Method number. The cursor will jump to another set of nested brackets on the same line. It is now possible to name your method.

p) To name your method CHARGE.RANGE.X:

Press the 9 key and then the ▲ key until a C is showing
Press the 9 key and then the ▲ key until an H is showing
Press the 9 key and then the ▲ key until an A is showing
Press the 0 key and then the ▼ key until an R is showing
Press the 9 key and then the ▲ key until a G is showing
Press the 9 key and then the ▲ key until an E is showing
Press the 0 key and then the ▼ key until a "." is showing
Press the 0 key and then the ▼ key until an R is showing
Press the 9 key and then the ▲ key until an A is showing
Press the 0 key and then the ▼ key until an N is showing
Press the 9 key and then the ▲ key until a G is showing
Press the 9 key and then the ▲ key until an E is showing
Press the 0 key and then the ▼ key until a "." is showing
Press the 0 key and then the ▼ key until an X is showing

By now your screen should look like Figure 12-57 below.

```
                SAVE METHOD
Save as method # (3){(CHARGE.RANGE.X)}
New method mode: Undefined Date:----
[CANCEL]            [PREVIOUS]  [SAVE]
```

Figure 12-57: The Save Method Screen with Method Name Added

Now press the [ENTER] key and then the [SAVE] key.

Messages about the saving process will be displayed and finally you will see the METHOD STORED screen as depicted here as Figure 12-58.

```
               METHOD STORED
Saved as method 3.
Select the NEXT key to continue
                                  [NEXT]
```

Figure 12-58: The Method Stored Screen

The [NEXT] key will return you to the COULOCHEM MAIN MENU.

NOTE: Occasionally after saving a timeline step the [NEXT] key is pressed instead of the [ENTER] key. When this happens you will exit the Edit/Create Timeline Event screen and be asked to save your method. Simply press the [PREVIOUS] key to get back to where you were when you inadvertently pressed [NEXT].

Hopefully these last two examples have helped you understand the potential utility of the Timeline mode and how it is implemented.

13 Scan Mode Operation

NOTE: Scan Mode operation is provided via the optional Pulse/Scan Potentiostat Board (Pulse/Scan Upgrade Part Number 70-5504) and requires a Model 5040 or Model 5041 Analytical Cell. Other ESA cells will not provide meaningful data. In addition, an X-Y recorder or equivalent to display the potential and the current is required.

13.1 Overview of Scan Mode Operation

In Scan Mode, the potential of the cell is changed as a function of time to obtain a plot of the oxidative (reductive) current vs. the potential for the compound(s) of interest to generate a cyclic voltammogram (see Section 13.2). This information is useful for estimating the potential for a given chromatographic analysis and is especially useful when the sample is quite simple.

Hydrodynamic Voltammetry (HDV) is an alternative to cyclic voltammetry for determining the appropriate potential(s) for the analysis of the various compounds of interest in a sample. While this technique does not formally employ Scan Mode, we discuss it below (Section 13.3) as it provides similar information as cyclic voltammetry.

13.2 Cyclic Voltammetry

If the current from a given sample at a constant concentration is measured over a range of potentials, a cyclic voltammogram can be obtained. An example of a cyclic voltammogram from a solution of 0.10 N NaOH using a gold electrode is shown in Figure 13-1. For optimum results, the Model 5040 Analytical Cell with the appropriate target should be used with Scan Mode.

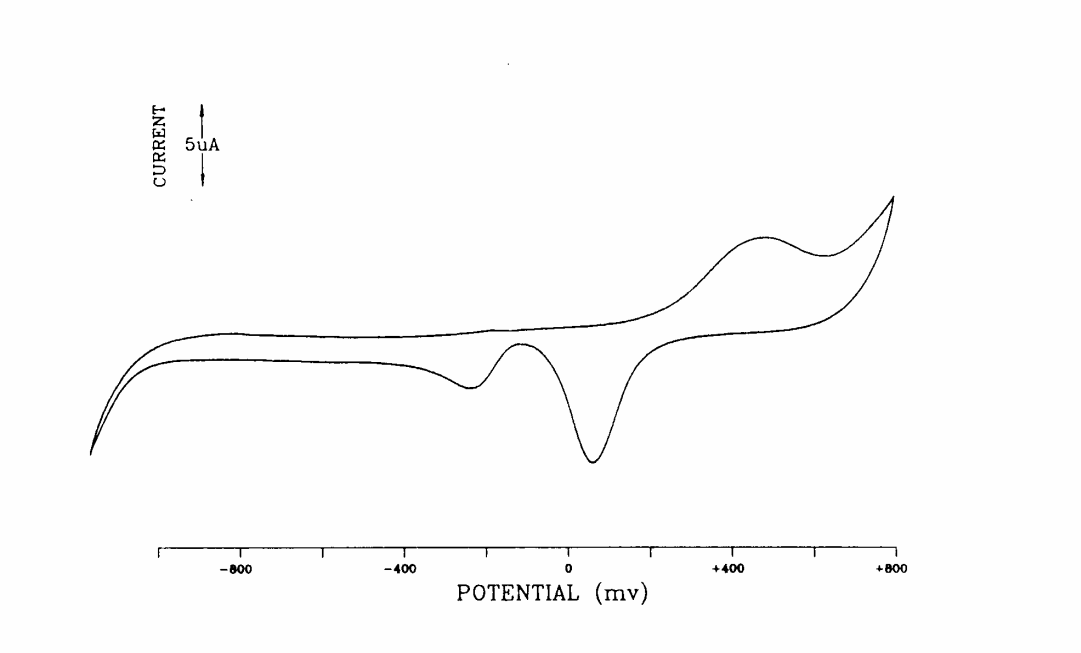

Figure 13-1: Cyclic Voltammogram of 100 mN NaOH Using a Gold Electrode with a Model 5040 Analytical Cell

Cyclic voltammetry provides very useful information that assists in the determination of the appropriate potential required for the analysis of a compound of interest. Since the cyclic voltammogram includes information about both the oxidation and reduction of a sample, it is possible to determine if an electrochemical process is reversible. In addition, the method can provide information about possible multi-step reactions and about the electrochemical properties of mixtures of electroactive species.

Ideally, a cyclic voltammogram is collected for each compound to be analyzed (e.g., by stop-flow injection of the compound directly into the flow cell). These voltammograms then provide the information to set the potential to quantitate each compound. The localized current maxima in the cyclic voltammogram indicates the potential that should be used. It should be noted that the concentration of the compound of interest in the cell may change during the collection of the cyclic voltammogram.

NOTE: The concentration of the analyte needed for cyclic voltammetry is much higher than is needed for detection in DC mode. Typically, the concentration of the analyte of interest needs to be about 1 mM.

If the sample contains more than one compound, a peak in the cyclic voltammogram may be observed for each compound at the appropriate potential (provided, of course, that there is a sufficient potential difference between two compounds that have similar oxidation (or reduction) potentials). In Figure 13-2, the cyclic voltammogram from a mixture is presented; the voltammogram clearly indicates that electroactive compounds are present in the sample. (Although the cyclic voltammograms of compounds can be quite complex and difficult to interpret, assume that the voltammogram in Figure 13-2 is made up of the response from three separate compounds.)

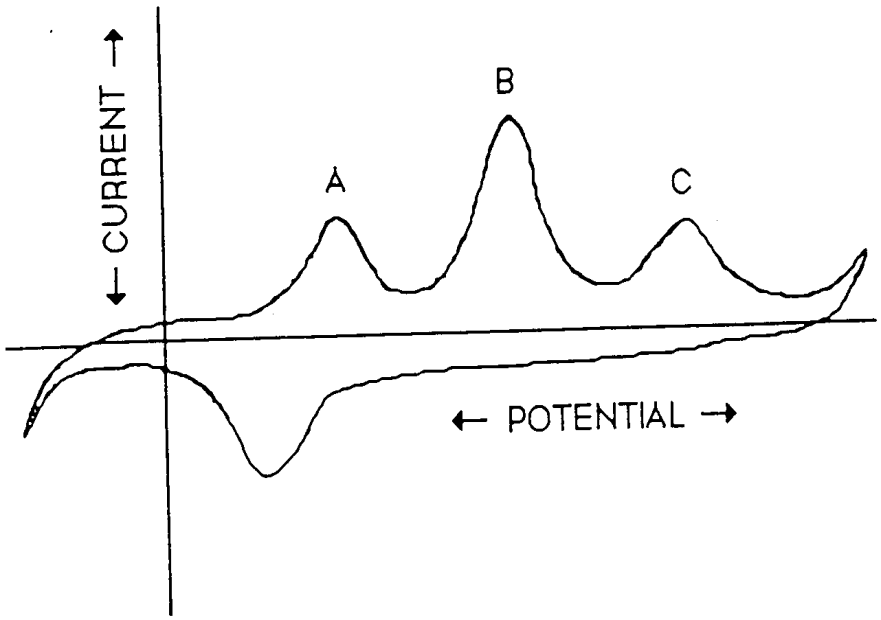

Figure 13-2: Cyclic Voltammogram of a Mixture Containing Electroactive Compounds

Additionally, it can be seen that to detect compound A, a potential should be used that is greater than the peak potential of A, but not greater than the valley between peaks A and B. A similar reasoning can be applied for analyzing for B and C.

A significant problem in cyclic voltammetry is the fact that capacitive currents occur as the applied potential is changed during the scan. These currents occur in addition to the current from the actual electrochemical process and can distort the desired data. The capacitive current increases as the scan rate increases, thus there is a practical limit to the scan rate.

In some cases, the use of cyclic voltammetry can result in the loss of important information. In a two electrode system, some compounds may undergo both an oxidation and a reduction in the millisecond time frame when the sample is flowing through the cell. However, if a single cell is used, the reaction might not appear to be reversible within the limited scan rate, due to the slow scan rate needed to cycle between the potential limits.

The parameters used to establish Scan Mode operation are described in Table 13-1 and a detailed discussion on how a method is setup is presented in Section 13-4.

Table 13-1: Scan Mode Parameters

Parameter	Description	Range	Default
X-Axis Output Divisor	Selects the range for the potential (X-axis) so that the scan appears full scale on the XY recorder	1-10 (step increments)	1
Current Range	Used to set the sensitivity level of the detector to ensure peaks are on scale	1 nA to 10 mA (step increments)	100 µA
Number of Cycles	Number of times that scan is to be run	1-9999 (integer increments)	2
Scan Rate	Rate of change of the potential	1 to 100 mV/sec (step increments)	2 mV/sec
Initial Potential	Potential to be used for starting of scan	-2000 to +2000 mV (integer increments)	0 mV
Potential Limit 1	Potential to be scanned to (e.g., upper potential)	-2000 to +2000 mV (integer increments)	50 mV
Potential Limit 2	Second potential to be scanned to (e.g., lower potential)	-2000 to +2000 mV (integer increments)	-50 mV
Final Potential	Potential to be used for end of scan	-2000 to +2000 mV (integer increments)	0 mV
Voltage Output (Record Out)	Indicates the voltage that is provided by the detector to drive the recording device	+1 V to -1 V (step increments)	+1 V
Filter (not implemented)	Smoothing function to reduce noise	None	None

13.3 Hydrodynamic Voltammetry

Hydrodynamic voltammetry can be used to generate a current voltage (CV) curve or HDV as an alternative to cyclic voltammetry. In this mode, a constant amount or a constant concentration of the sample is injected into the HPLC system and separated (typically the on-column sample should be fairly concentrated, e.g., from 10 to 100 ng of the analyte). The Model 5010 Standard Analytical Cell, the Model 5011 High Sensitivity Analytical Cell, or the Model 5014B Microdialysis Cell can be used for hydrodynamic voltammetry as well as the amperometric cells (Model 5040, 5041). The detector is set at a given potential and the chromatogram is collected. The peak height (or peak area) is measured for each compound in the sample. The process is repeated with varying potentials until sufficient data is collected, and a peak height (area) vs. applied potential curve is generated.

There are a number of advantages to hydrodynamic voltammetry including:

- The observed current does not include a contribution from capacitive effects, since the potential is allowed to equilibrate before the data is collected.
- It is not necessary to replumb the HPLC.
- There is a higher degree of certainty that a given peak is due to one and only one compound, since a chromatographic separation is often used in conjunction with the method.
- Short lived species formed by the redox technique can be studied (this is not possible via cyclic voltammetry). Since the flow rate can be controlled, hydrodynamic voltammetry provides an added degree of flexibility.

While the amount of time required to construct a hydrodynamic voltammogram is considerably longer than that required for a cyclic voltammogram, a more accurate (and more useful) description of the electrochemical properties of the sample are obtained.

As a time-saving measure, the collection of the data for a hydrodynamic voltammogram can be automated using the Coulochem® III detector with an ESA data station or equivalent and associated software or the use of the CV curve function of TIMELINE mode can be used with any recording system (see sections 4.2.1 and 9.4).

ESA recommends generating a CV curve utilizing hydrodynamic voltammetry rather than using cyclic voltammetry.

☞ NOTE: Chromatographic conditions must be established before performing hydrodynamic voltammetry. A change in the chromatographic conditions could affect the optimal analysis potential.

13.4 Generating a Scan Method

To set Scan Mode operations:

a) Access the EDIT MODE SELECTION Screen (Figure 13-3), by pressing [EDIT] on the COULOCHEM MAIN MENU.

```
          EDIT MODE SELECTION
Method Number: (1) ""
Mode:DC        Date: Jun 26,2002 13:57
[CANCEL]                       [EDIT]
```

Figure 13-3: The Edit Mode Selection Screen

b) Press [EDIT] to access the MODE SELECTION Screen (Figure 13-4). The cursor will be in the New Mode field.

```
             MODE SELECTION
This method's Mode is DC
New Mode is (DC) with (2) Channel(s)
[CANCEL]             [PREVIOUS] [NEXT]
```

Figure 13-4: The Mode Selection Screen

c) Move the ▲or ▼button until SCAN is indicated and press ENTER. After the screen has been edited, press [NEXT] to the SCAN EDIT Screen #1 (Figure 13-5).

```
                SCAN EDIT
Init Potl {0}mV     Final Potl{0}mV
Potl Limit 1(50)mV     Limit 2{-50}mV
[CANCEL]                     [NEXT]
```

Figure 13-5: The Scan Edit Screen #1

d) Edit the parameters on the SCAN EDIT Screen #1. A detailed discussion of each parameter, the range for each parameter and the mode of editing is presented in Table 13-1.

☞ NOTE: The general approach for entering values in Scan Mode is identical to that for DC Mode. As an example, if you want to edit the Intl Potl, move the cursor to that field and enter the desired value via the keypad. After you have entered the parameter, press ENTER to move the cursor.

e) After you have completed editing the SCAN EDIT screen #1, press [NEXT] to access and edit SCAN EDIT Screen #2 (Figure 13-6).

```
                SCAN EDIT
Current Range R(100uA)   X Divisor (1)
Output {1.0}V   Filter (None)
[CANCEL]            [PREVIOUS]    [NEXT]
```

Figure 13-6: The Scan Edit Screen #2

f) After you have completed editing the SCAN EDIT screen #2, press [NEXT] to access edit SCAN EDIT Screen #3 (Figure 13-7).

```
                SCAN EDIT
Scan Rate {2} mV/Sec
Number of Cycles {2}
[CANCEL]       [PREVIOUS]    [NEXT]
```

Figure 13-7: The Scan Edit Screen #3

g) After you have completed editing the SCAN EDIT screen #3, press [NEXT] to access the SAVE METHOD Screen (Figure 13-8), which is used to store the method in memory. When the screen is opened, the cursor is placed on the method number.

```
               SAVE METHOD
Save as method #(2) {()}
New method mode: Undefined   Date:----
[CANCEL]          [PREVIOUS]    [SAVE]
```

Figure 13-8: The Save Method Screen

The method number is the number assigned at the start of the editing of the method, but can be changed via the ▲ or ▼ buttons. After the method number has been selected, press ENTER. The cursor will move to the {()}field and you can enter additional information about the method (i.e., name of the method, your initials, etc.). The date and time will be automatically entered when you save the method.

Press [SAVE] to save the method. The method will be stored in memory and a prompt will be presented to press [NEXT] to continue.

13.5 Initiating and Performing a Scan

Scan mode operation can be initiated by opening a scan method on the SELECT METHOD Screen (Figure 13-9) and pressing [RUN].

```
                SELECT METHOD
Method Number: (5) "CATS"
Type: Scan    Date: Jan. 3, 2002 13:44
[CANCEL]                          [RUN]
```

Figure 13-9: The Select Method Screen

When the [RUN] button is pressed on the Run Method Selection screen, the method will be started and the SCAN METHOD 1 HOLDING Screen #1 (Figure 13-10) will be presented.

```
          SCAN METHOD  1  HOLDING
E{100} mV    i: 20.13 uA    %FS: 40
EL1{10 mV}   Cycles {1}  EL2{0}mV
[STOP]    [REVERSE]    [RUN]    [SCREEN2]
```

Figure 13-10: The Scan Method 1 Holding Screen #1

In Figure 13-10, **E** is the Initial Potential
i is the present current
% FS is the percent full scale for the present current
EL1 is the initial potential to be scanned to (e.g., upper potential)
Cycles is the number of cycles to be performed
EL2 is the second potential to be scanned to (e.g., lower potential)

The buttons on the Scan Method Running screen provide the following:

[STOP] halts the present scan
[REVERSE] the scan will operate in the reverse manner
[RUN] initiates the scan method (the button will change to [HOLD]).
[SCREEN2] accesses additional scan parameters (Figure 13-11)

```
          SCAN METHOD  1  HOLDING
Range (100 uA) Xdiv (   1) Filt(None)
Rate {10} mV/Sec Record out (  1.0)
[STOP]    [REVERSE]   [RUN]   [SCREEN1]
```

Figure 13-11: The Scan Method 1 Holding Screen #2

The potentials (EL1, EL2 and E), Cycle Number, Current Range, Record Out and Scan Rate can be edited by first pressing the ENTER key and editing as normal. The cursor can be moved to the appropriate field by repeatedly pressing the ENTER key. Parameters can be changed using the arrow keys or numeric keypad as appropriate.

NOTE: Changing the parameters during a run will not change the method on a permanent basis. If you want to save the changes, it will be necessary to edit the method as described in Section 13.4.

When you press the [RUN] key, the scan will be started and will proceed as programmed. The Cycles field will indicate the number of scans that have not yet been completed. The detector will automatically stop scanning at the end of the program or if you press the [HOLD] or [STOP] button.

NOTE: Be sure that the CELL ON/OFF is set to ON, otherwise no potential will be applied to the electrodes.

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14 Coulochem III Organizer Modules

14.1 Overview

The *Coulochem® III Organizer Module* and the *Coulochem® III Thermal Organizer Module* are optional accessories for the *Coulochem III Electrochemical Detector* that are designed to accommodate the various components of the fluidics system such as electrochemical cells, pulse damper, column(s), in-line filters, a Rheodyne manual sample injector valve, tubing, cables, etc. The organizer module is mounted on top of the detector housing as shown in Figure 1-1 of the *Coulochem III (50W) User's Guide Manual*. The *Coulochem III Thermal Organizer Module* allows the analyst to maintain the temperature of the column between ambient + 5°C and 60°C (the *Coulochem III Organizer Module* does not include the ability to change the temperature of the column).

A detailed discussion of the connection of the various components of the HPLC fluidics system is presented in Chapter 3 of the *Coulochem III (50W) Reference Manual.* This appendix describes how the components are mounted in the modules as well as maintenance and cleaning instructions. When either organizer module is included in the system, each component should be installed in the same order as described in Chapter 3. In addition, the fluidics system should be tested in the same manner as is described in Chapter 3.

The components that are to be mounted in the module are determined by the application for which the detector will be used. A typical organizer configuration might include a manual sample injector, a pulse damper, an in-line filter before the column, an HPLC column, an in-line filter before the electrochemical cells and two electrochemical cells.

14.2 Unpacking the Organizer

The organizer module is shipped in a single carton containing the module and an accessory kit. Unpack the shipment and inspect the contents to verify receipt of all components. A *Customer Inventory Checklist*, which lists the parts shipped with the organizer, is presented as Table 14-1 for the *Coulochem III Organizer Module* and Table 14-2 for the *Coulochem III Thermal Organizer Module*.

Carefully inspect the shipping carton and all components. If there is any damage to the carton or to any components, contact both the shipping agent and ESA (or its representative) immediately. If any parts are missing, call ESA's customer service department and indicate the missing items via the part numbers. The shipping carton should be retained as it can be used if it becomes necessary to transport the organizer.

The module should be placed in an area that is free from drafts or significant temperature changes. Avoid placing the organizer near air conditioning vents, windows, ovens, etc.

Table 14-1: Accessory Kit - Coulochem® III Organizer Module

Quantity	Item	Part Number
2	8-32 x 3/8 Screw and Star Washer for Manual Injector	50-5213
16	Feet for Cells	70-4336
1	Organizer Ground Strap	70-5625
1	Column Grounding Cable	70-5630
6 feet	Tubing for Drain	50-6224
1	Bracket for Manual Injector Overflow Line	70-5678
2	Bracket for Holding Down Cells	70-5439
4	Plastic Screws for Cell Bracket	70-5571
3	Knurled Screws for Cell Bracket	70-5564
1	Panel with Hole for Manual Injector	70-5229
1	Panel without Hole for Automated Injector	70-5418
2	Knurled Thumbnuts for Panels	70-5532
10	Weigh Boats for Injector Waste	40-0172

Table 14-2: Accessory Kit - Coulochem® III Thermal Organizer Module

Quantity	Item	Part Number
2	8-32 x 3/8 Screw and Star Washer for Manual Injector	50-5213
16	Feet for Cells	70-4336
1	Organizer Ground Strap	70-5625
1	Column Grounding Cable	70-5630
2	Column Holder Assembly	70-5530
6 feet	Tubing for Drain	50-6224
1	Bracket for Manual Injector Overflow Line	70-5678
2	Bracket for Holding Down Cells	70-5439
4	Plastic Screws for Cell Bracket	70-5571
3	Knurled Screws for Cell Bracket	70-5564
1	Panel with Hole for Manual Injector	70-5229
1	Panel without Hole for Automated Injector	70-5418
2	Knurled Thumbnuts for Panels	70-5532
10	Weigh Boats for Injector Waste	40-0172
1	Cable Interface Thermal Organizer, 50 Watt	70-5595A

14.3 Installing the Coulochem III Organizer Module

☞ NOTE: Refer to Section 14.4 to install the Coulochem® III Thermal Organizer Module

14.3.1 General Information

Cut the tubing for each connection and prepare the appropriate fittings before installing the various components into the organizer. The tubing length should be long enough to allow for a bit of play, but excessive length should be avoided (especially after the HPLC injector except for the waste tubing exiting the last electrochemical cell). It is a good idea to use a heat gun or similar device to heat PEEK tubing in order to bend it without unduly stressing it. If PEEK tubing is stressed it becomes weakened and can either burst at lower pressures than expected or leaks or "sweating" may occur. This is especially important when sharp bends are used (sharp bends can be found in many configurations or applications used with the Coulochem III and the Organizer Module).

Tubing to and from other components in the system (e.g., the pump) can be led into the organizer via the vertical cuts in the cover (Section 14.3.2).

An exploded view of the entire Coulochem III Organizer Module is shown in Figure 14-1. Please refer to this figure as you assemble the Organizer Module.

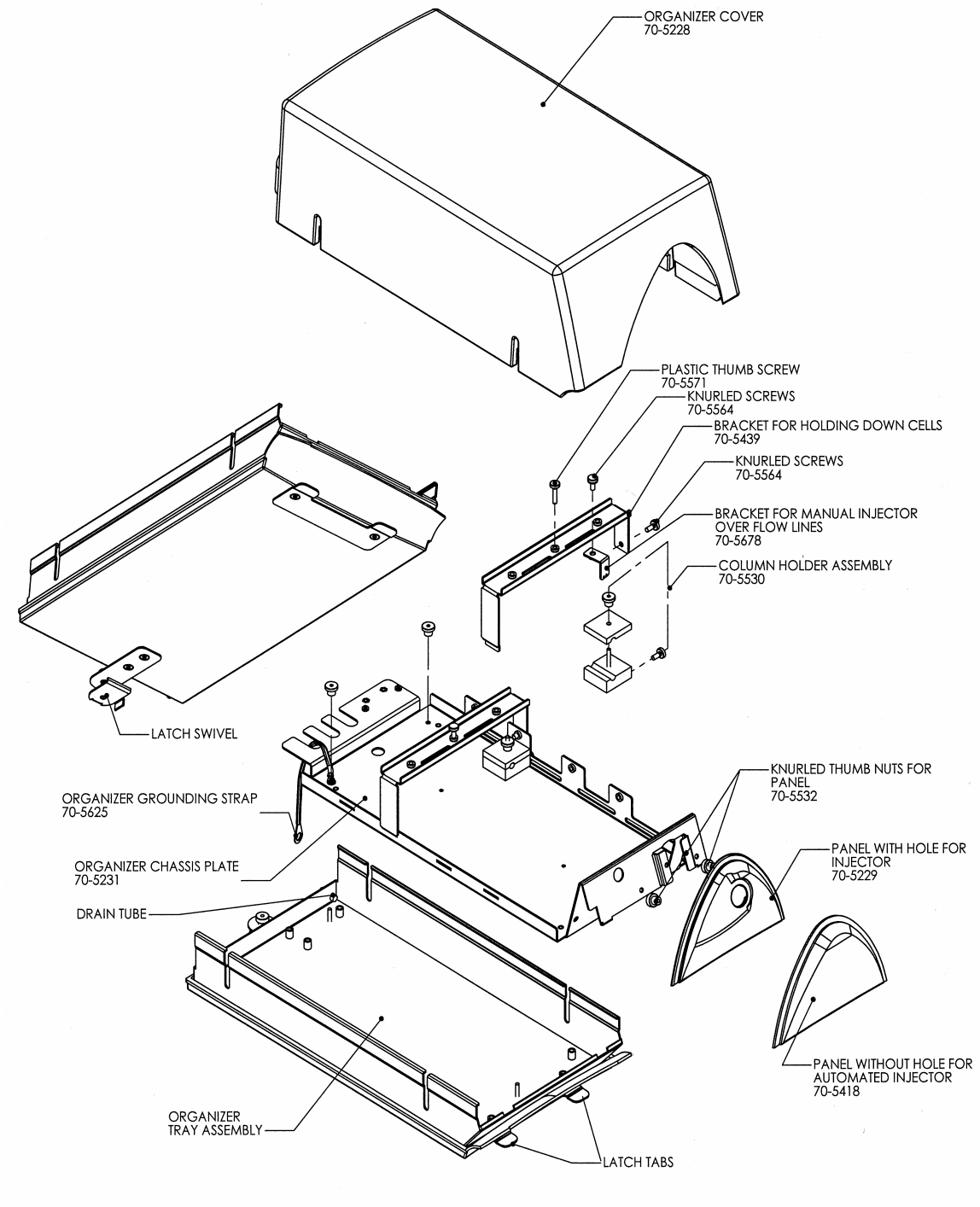

Figure 14-1: Exploded View of Organizer Module

14.3.2 Removing the Organizer Chassis Plate

All components are mounted on the *Organizer Chassis Plate* (Part Number 70-5231; Figure 14-2), which is attached to the *Organizer Tray*. It is necessary to remove the chassis plate from the organizer tray to attach the various components.

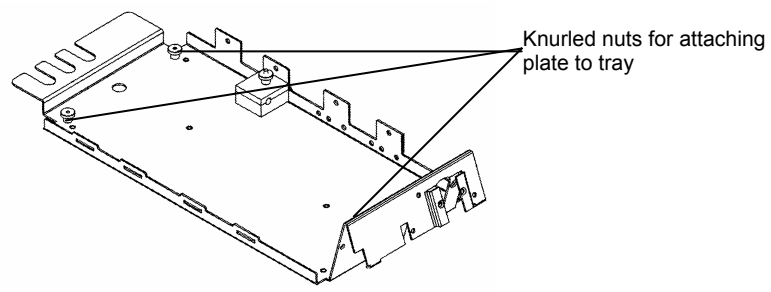

Figure 14-2: Organizer Chassis Plate

To remove the Organizer Chassis Plate:

a) Remove the Organizer Cover by lifting it off.

b) Unscrew the three knurled nuts that attach the organizer chassis plate to the tray. One nut is in the front (center) and the other two are in the corners at the rear of the organizer.

c) Lift the organizer tray from the chassis plate.

14.3.3 Installing the Pulse Damper

To install the Pulse Damper:

a) The pulse damper is mounted on the rear of the front left side of the chassis plate (Figure 14-3) using 2 of the 4 screws that are supplied with the pulse damper.

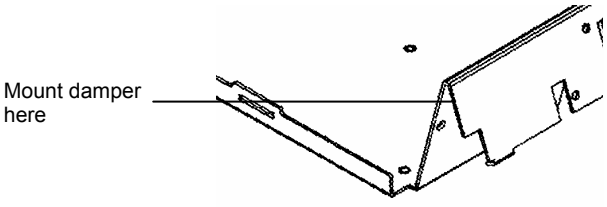

Figure 14-3: Mounting the Pulse Damper

☞ NOTE: Do not attach the rubber feet to the bottom of the pulse damper when mounting it to the chassis plate.

14.3.4 Installing a Rheodyne Manual Sample Injector Valve

☞ NOTE: It may be easier to connect the appropriate tubing to the manual sample injector valve before installing it into the organizer.

To install the Manual Sample Injector:

a) Place the Organizer Injector Panel with the hole (Part Number 70-5229) onto the organizer chassis plate (Figure 14-4).

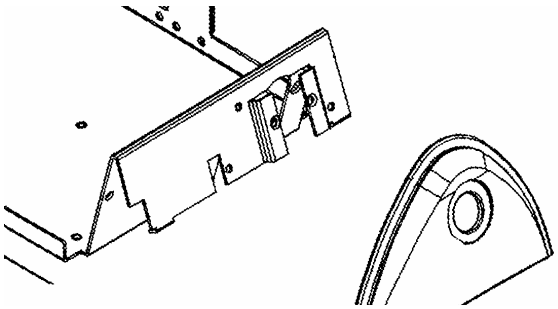

Figure 14-4: Locating the Organizer Injector Panel

b) Affix the panel to the organizer chassis plate with the two thumbnuts provided.

c) Remove the handle from the injector valve using a hex wrench.

d) Attach the injector to the chassis so that the valve stem goes through the hole in the Injector Panel and the *part number* of the injector is *facing up*. Fasten the injector using the two screws and star washers (Part Number 50-5213) supplied in the accessory kit.

☞ NOTE: The screws and star washers are provided to ensure appropriate grounding. Do not use the screws supplied with the injector to mount the injector.

e) Reattach the injector handle and tighten the two hex setscrews. Make sure the setscrews make contact with the flat portions of the valve stem. It will be necessary to rotate the handle after tightening one setscrew to gain access to the other setscrew.

Refer to the Operating Instructions provided with the injector for other information on installation, proper use and care of the manual sample injector valve. These instructions include information about the wiring for the position-sensing switch (optional) that can be connected to another HPLC component, which is used to signal the start of the injection. The cabling for the position sensing switch can be directed outside of the organizer at the back of the organizer along with the cell cables.

If an autosampler is to be used:

a) Place the Organizer Injector Panel without the hole (Part Number 70-5418) onto the organizer chassis plate.

b) Affix the organizer injector panel to the organizer chassis plate with the two thumbnuts.

c) Lead in the tubing from the autosampler through one of the ports on the proper side of the organizer.

14.3.5 Installing the In-line Filter

If an in-line filter is employed, it should be connected between the injector and the column. The filter holder can be allowed to sit on the floor of the chassis plate.

14.3.6 Installing the Column Holder

The column is clamped to the chassis plate using a Column Holder Assembly (Part Number 70-5530) that is mounted to the right side of the chassis. The holder is assembled as shown in Figure 14-5 and is mounted to the side of the chassis via the lower set of screw holes on the right side of the chassis as shown in Figure 14-6.

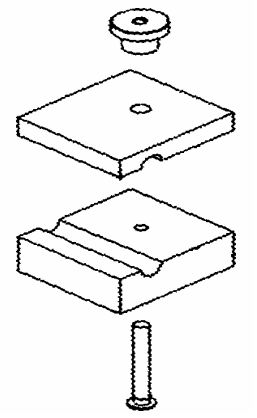

Figure 14-5: Column Holder Assembly

One holder should be used for short columns, and two holders can be used for longer columns.

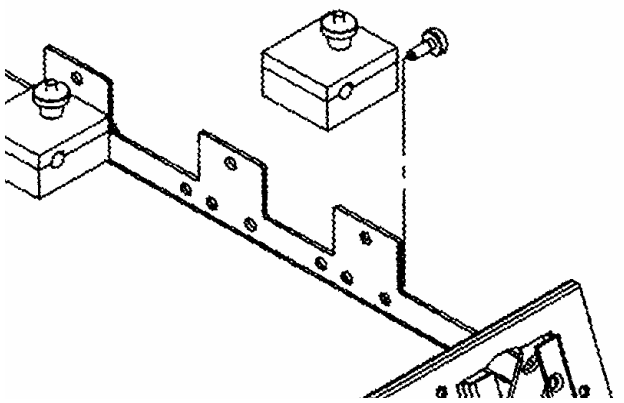

Figure 14-6: Mounting the Column Holder Assembly

NOTE: The Column Grounding Cable (Part Number 70-5630) can be used to ground the column to the outlet of the analytical cell. In high sensitivity analyses, the use of the column grounding cable placed between the column and the outlet of the cell (especially the Model 5041 Cell) may result in reduced baseline noise.

14.3.7 Electrochemical Cells

Electrochemical cells can be placed as desired on the base on the organizer chassis plate. After the cell(s) are located, they can be secured using the Cell Brackets (Part Number 70-5439) that are provided. Connect the brackets to the left side of the chassis by the lip that fits into the horizontal slit and the knurled screw that fits into the right side of the chassis (Figure 14-7). The plastic knurled screws (Part Number 70-5571) should be threaded into the proper hole over a cell and secured finger tight.

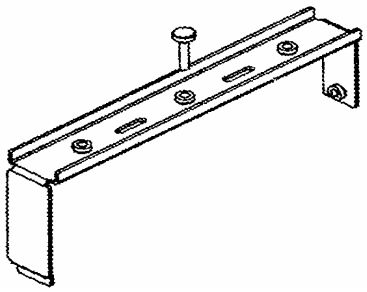

Figure 14-7: Cell Bracket

Rubber feet (Part Number 70-4336) are provided to provide a secure connection between the cells and the chassis plate. The feet can be placed on either side of the cells, which can then be placed on the chassis plate, to help facilitate fitting and orienting the cells into the Organizer Module in an optimum manner. The feet can also help prevent any leaked mobile phase from entering into a cell if it is mounted on its side.

The electrochemical cells should be mounted so that both the cables can be directed out of the organizer through the slots at the rear of the chassis plate and the tubing connecting the cells (inlet and outlet) can be guided to the proper connection (column, in-line filter, etc.).

14.3.8 Manual Sample Injector Overflow Line Bracket

The Manual Injector Overflow Line Bracket is an L-shaped piece with small holes for tubing (Part Number 70-5678). It should be mounted to the underneath side of one of the cell brackets. The overflow lines (vent tubes) from the injector valve should be positioned through the small holes so that any overflow can drip into a Weigh Boat (Part Number 40-0172) that is placed on the organizer chassis plate. The overflow bracket is designed to keep the outlet of the vent tubes at the same vertical height as that of the needle port to prevent siphoning of the sample.

14.3.9 Final Assembly

After you have made all connections:

a) Place the chassis plate into the organizer tray so that the three threaded studs protrude through the bottom of the chassis plate.

b) Connect the Ground Strap Assembly (Part Number 70-5625) to the protruding threaded stud at the left-rear corner of the chassis plate (see Figure 14-1). Attach the three knurled thumbnuts, which were removed in Section 14.3.1, and finger tighten.

c) Mount the organizer module on top of the Coulochem III by inserting the latch tabs at the front of the organizer into the two slots at the top back of the detector's bezel. Make sure the swivel plate of the latch assembly at the rear of the organizer is underneath the top lip of the detector. Then tighten the latch using the knurled thumbnut.

   In most cases, the organizer is attached to the top of the Coulochem III detector, but it can also be placed on the bench next to the Coulochem III if so desired. Remove the latch mechanism at the rear of the organizer so the organizer will set flat on the bench. The organizer must also be set as close as possible to the detector to ensure that the cell cables will readily reach it.

d) Connect the loose end of the Ground Strap Assembly to the ground screw on the back of the Coulochem III. It is located in the upper right hand corner (when facing the back of the detector) of the power supply module next to the ground symbol (see page v and Figure 2-1 in this manual).

☞ NOTE: The Ground Strap that is provided will not reach to the Coulochem III when the organizer is placed next to it on a bench. The user must provide a grounding cable for this configuration.

e) Connect the tubing for the drain onto the protruding tube on the right rear of the organizer. Place the other end of the drain tubing in a waste container that is situated lower than the organizer.

f) Route the cell cables out the back of the organizer using the slots in the chassis plate located at the rear of the organizer. Connect cell cables to the proper connectors on the rear panel of the Coulochem III.

g) Replace the cover.

h) Make all the necessary connections to your HPLC system.

Now you are ready to run your application.

14.4 Installing the Coulochem III Thermal Organizer Module

☞ NOTE: Refer to Section 14.3 to install the Coulochem III Organizer Module.

14.4.1 General Information

Cut the tubing for each connection and prepare the appropriate fittings before installing the various components into the organizer. The tubing length should be long enough to allow for a bit of play, but excessive length should be avoided (especially after the HPLC injector except for the waste tubing exiting the last electrochemical cell). It is a good idea to use a heat gun or similar device to heat PEEK tubing in order to bend it without unduly stressing it. If PEEK tubing is stressed it becomes weakened and can either burst at lower pressures than expected or leaks or "sweating" may occur. This is especially important when sharp bends are used (sharp bends can be found in many configurations or applications used with the Coulochem III and the Thermal Organizer Module).

Tubing to and from other components in the system (e.g., the pump) can be led into the organizer via the vertical cuts in the cover (Section 14.4.2).

An exploded view of the entire Coulochem III Thermal Organizer Module is shown in Figure 14-8. Please refer to this figure as you assemble the Organizer Module.

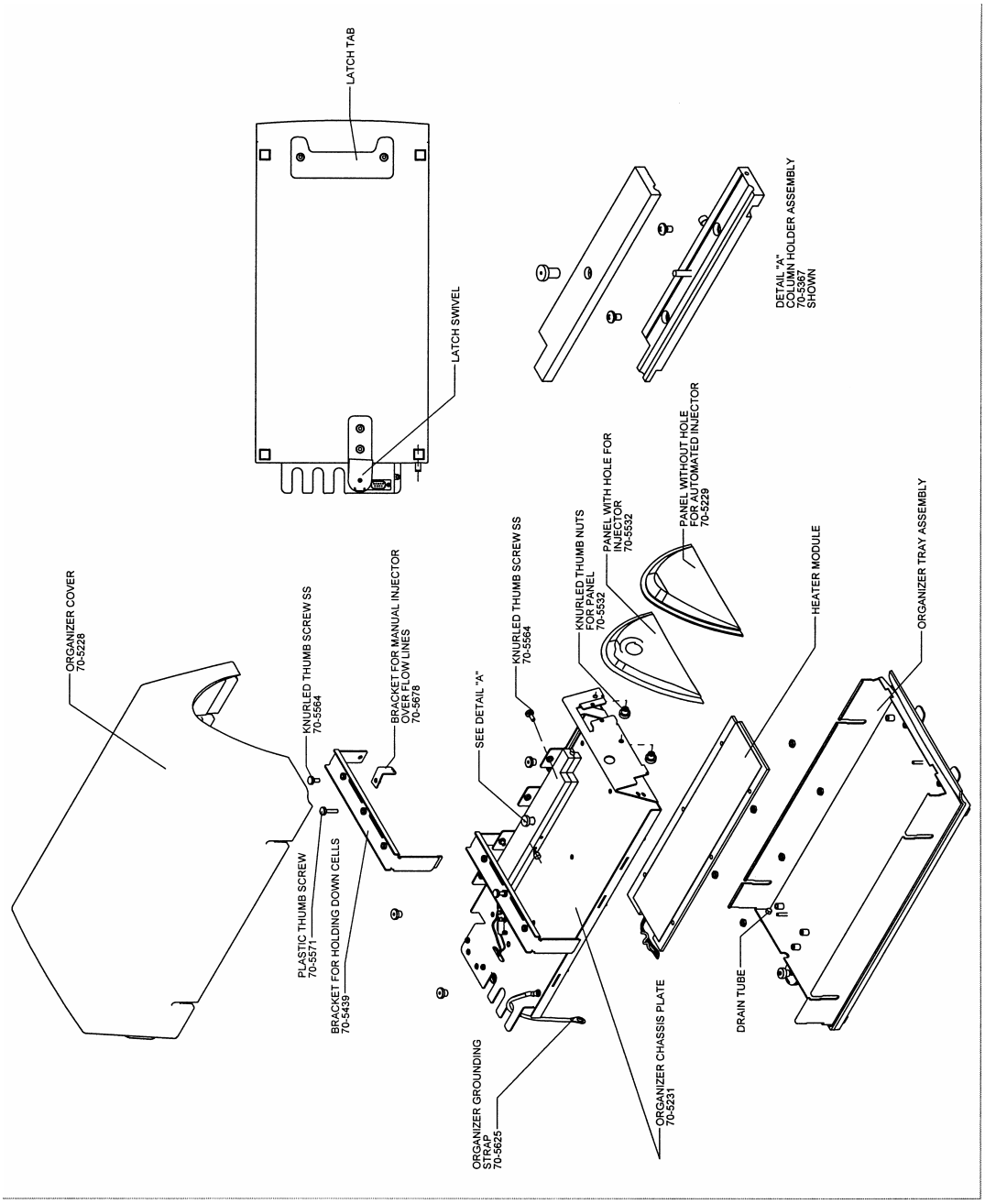

Figure 14-8: Exploded View of Organizer Module

14.4.2 Removing the Organizer Chassis Plate

All components are mounted on the *Organizer Chassis Plate* (Part Number 70-5231; Figure 14-9), which is attached to the *Organizer Tray*. It is necessary to remove the chassis plate from the organizer tray to attach the various components.

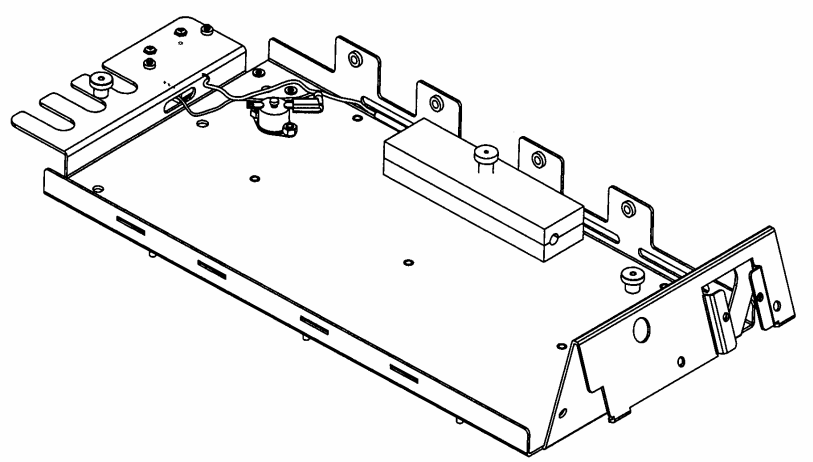

Figure 14-9: Organizer Chassis Plate

To remove the Organizer Chassis Plate:

a) Remove the Organizer Cover by lifting it off.

b) Unscrew the three knurled nuts that attach the organizer chassis plate to the tray. One nut is in the front (center) and the other two are in the corners at the rear of the organizer.

c) Lift the organizer tray from the chassis plate.

14.4.3 Installing the Pulse Damper

The pulse damper is mounted on the rear of the front left side of the chassis plate (Figure 14-10) using 2 of the 4 screws that are supplied with the pulse damper.

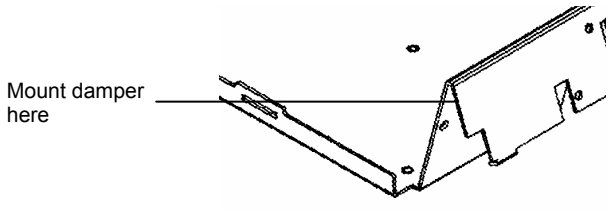

Figure 14-10: Mounting the Pulse Damper

☞ NOTE: Do not attach the rubber feet to the bottom of the pulse damper when mounting it to the chassis plate.

14.4.4 Installing a Rheodyne Manual Sample Injector Valve

☞ NOTE: It is recommended that you connect the appropriate tubing to the manual sample injector valve before installing it into the organizer.

To install the Manual Sample Injector:

a) Place the Organizer Injector Panel with the hole (Part Number 70-5229) onto the organizer chassis plate (Figure 14-11).

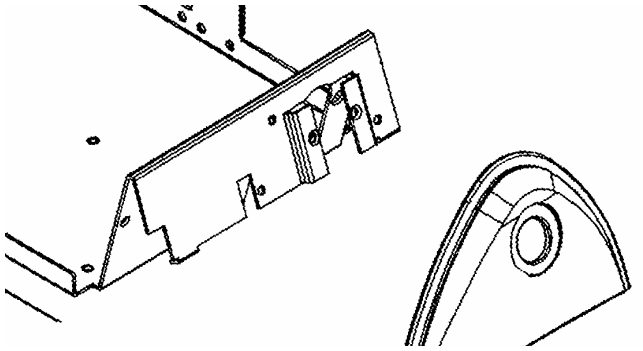

Figure 14-11: Locating the Organizer Injector Panel

b) Affix the panel to the organizer chassis plate with the two thumbnuts provided.

c) Remove the handle from the injector valve using a hex key.

d) Attach the injector to the chassis so that the valve stem goes through the hole in the Injector Panel and the *part number* of the injector is *facing up*. Fasten the injector using the two screws and star washers (Part Number 50-5213) supplied in the accessory kit.

NOTE: The screws and star washers are provided to ensure appropriate grounding. Do not use the screws supplied with the injector to mount the injector.

e) Reattach the injector handle and tighten the two hex setscrews. Make sure the setscrews make contact with the flat portions of the valve stem. It will be necessary to rotate the handle after tightening one setscrew to gain access to the other setscrew.

Refer to the Operating Instructions provided with the injector for other information on installation, proper use and care of the manual sample injector valve. These instructions include information about the wiring for the position-sensing switch (optional) that can be connected to another HPLC component, which is used to signal the start of the injection. The cabling for the position sensing switch can be directed outside of the organizer at the back of the organizer along with the cell cables.

If an autosampler is to be used:

a) Place the Organizer Injector Panel without the hole (Part Number 70-5418) onto the organizer chassis plate.

b) Affix the organizer injector panel to the organizer chassis plate with the two thumbnuts.

c) Lead in the tubing from the autosampler through one of the ports on the proper side of the organizer.

14.4.5 Installing the In-line Filter

If an in-line filter is employed, it should be connected between the injector and the column. The filter holder can be allowed to sit on the floor of the chassis plate.

14.4.6 Installing the Temperature Probe

The Temperature Probe (part number 70-5812) is placed in the access hole on the lower half of the column holder (Figure 14-12).

To install the Temperature Probe:

a) Loosen the clamping screw on the side of the column holder.

b) Slide the probe into the access hole until it is bottoms out.

c) Tighten the clamping screw against the probe.

d) Check to see that the probe is securely in place by slightly pulling back on it.

14.4.7 Installing the Column Holder

The Thermal Organizer is equipped with a 15 cm Column Holder Assembly (Part Number 70-5366) that is clamped to the chassis plate on the right side of the chassis. A 5 cm Column Holder Assembly (Part Number 70-5365) and a 25 cm Column Holder Assembly (Part Number 70-5367) are available as options.

The lower half of the column holder is mounted on the two short standoffs on the Organizer Chassis Plate. If it is necessary to remove the holder (e.g., to replace it or clean the organizer) remove the screws that attach the holder to the standoffs. When replacing the column holder, check that the holder sits flush to the chassis and that nothing is caught underneath, so that maximum contact is made.

As you install the column holder, take care that the cable from the sensor will not interfere with placing the column in the holder.

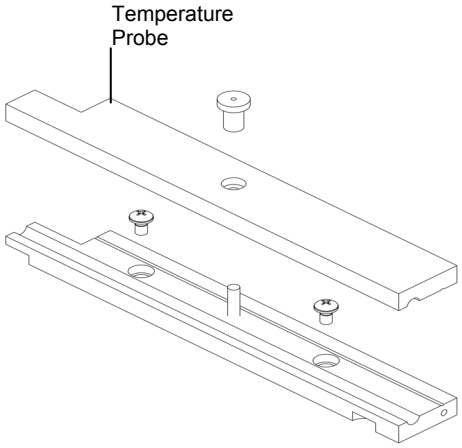

Figure 14-12: Column Holder Assembly

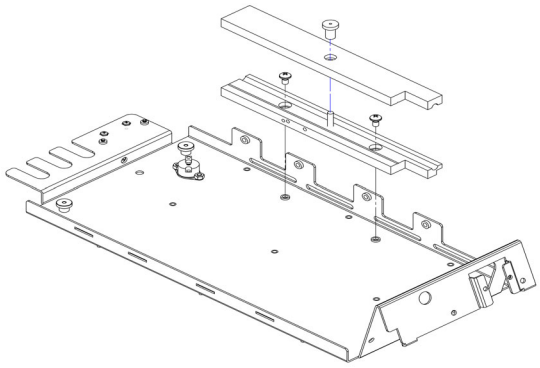

Figure 14-13: Mounting the Column Holder Assembly

NOTE: The Column Grounding Cable (Part Number 70-5630) can be used to ground the column to the outlet of the analytical cell. In high sensitivity analyses, the use of the column grounding cable placed between the column and the outlet of the cell (especially the Model 5041 Cell) may result in reduced baseline noise.

14.4.8 Electrochemical Cells

Electrochemical cells can be placed as desired on the base on the organizer chassis plate. After the cell(s) are located, they can be secured using the Cell Brackets (Part Number 70-5439) that are provided. Connect the brackets to the left side of the chassis by the lip that fits into the horizontal slit and the knurled screw that fits into the right side of the chassis (Figure 14-14). The plastic knurled screws (Part Number 70-5571) should be threaded into the proper hole over a cell and secured finger tight.

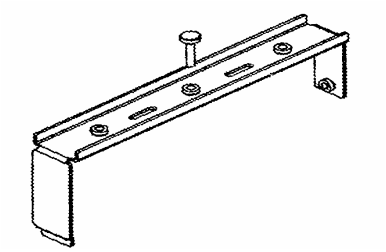

Figure 14-14: Cell Bracket

Rubber feet (Part Number 70-4336) are provided to provide a secure connection between the cells and the chassis plate. The feet can be placed on either side of the cells, which can then be placed on the chassis plate, to help facilitate fitting and orienting the cells into the Organizer Module in an optimum manner. The feet can also help prevent any leaked mobile phase from entering into a cell if it is mounted on its side.

The electrochemical cells should be mounted so that both the cables can be directed out of the organizer through the slots at the rear of the chassis plate and the tubing connecting the cells (inlet and outlet) can be guided to the proper connection (column, in-line filter, etc.).

14.4.9 Manual Sample Injector Overflow Line Bracket

The Manual Injector Overflow Line Bracket is an L-shaped piece with small holes for tubing (Part Number 70-5678). It should be mounted to the underneath side of one of the cell brackets. The overflow lines (vent tubes) from the injector valve should be positioned through the small holes so that any overflow can drip into a Weigh Boat (Part Number 40-0172) that is placed on the organizer chassis plate. The overflow bracket is designed to keep the outlet of the vent tubes at the same vertical height as that of the needle port to prevent siphoning of the sample.

14.4.10 Final Assembly

After you have made all connections:

a) Place the chassis plate into the organizer tray so that the three threaded studs protrude through the bottom of the chassis plate.

b) Connect the Ground Strap Assembly (Part Number 70-5625) to the protruding threaded stud at the left-rear corner of the chassis plate (see Figure 14-8). Attach the three knurled thumbnuts which were removed in Section 14.4.1, and finger tighten.

c) Mount the organizer module on top of the Coulochem III by inserting the latch tabs at the front of the organizer into the two slots at the top back of the detector's bezel. Make sure the swivel plate of the latch assembly at the rear of the organizer is underneath the top lip of the detector. Then tighten the latch using the knurled thumbnut.

   In most cases, the organizer is attached to the top of the Coulochem III detector, but it can also be placed on the bench next to the Coulochem III if so desired. Remove the latch mechanism at the rear of the organizer so the organizer will set flat on the bench. The organizer must also be set as close as possible to the detector to ensure that the cell cables will readily reach it.

d) Connect the loose end of the Ground Strap Assembly to the ground screw on the back of the Coulochem III. It is located in the upper right hand corner (when facing the back of the detector) of the power supply module next to the ground symbol (see page v and Figure 2-1 in this manual).

NOTE: The Ground Strap that is provided will not reach to the Coulochem III when the organizer is placed next to it on a bench. The user must provide a grounding cable for this configuration.

e) Connect the Thermal Organizer Interface Cable, 50W (Part Number 70-5595A) between the thermal organizer, the Power Input Module and the Logic Module (the latter two modules are accessed via the rear of the detector chassis).

f) Connect the tubing for the drain onto the protruding tube on the right rear of the organizer. Place the other end of the drain tubing in a waste container that is situated lower than the organizer.

g) Route the cell cables out the back of the organizer using the slots in the chassis plate located at the rear of the organizer. Connect cell cables to the proper connectors on the rear panel of the Coulochem III.

h) Replace the cover.

i) Make all the necessary connections to your HPLC system.

Now you are ready to run your application.

NOTE: ESA electrochemical cells must be maintained below 45°C. If the temperature is raised above that limit, make sure that the cell temperature is kept below 45°C. It may be necessary to place the cells outside of the Thermal Organizer.

14.5 Troubleshooting

The left part of the top line of the Main screen presents the temperature of the Thermal Organizer. In the event that a fault is observed in the organizer, the field will indicate a negative number as a code to indicates the nature of the fault and the help button can be used to present the text associated with the error code.

The codes are indicated in Table 14-3.

Table 14-3: Error Messages - Thermal Organizer

Code	Message	Suggested Action
0	Thermal Organizer not detected	If installed, check Temperature Probe connections
-1	Thermal Organizer malfunction	Check Temperature Probe connections
-2	Thermal Organizer malfunction (logic)	Check Temperature Probe connections
-3	Temperature out of range (under)	Check Temperature Probe and set point
-4	Organizer sensor malfunction	Check Temperature Probe connections
-5	Thermal Organizer malfunction (logic)	Check Temperature Probe connections
-6	Temp sensor or heater not responding	Check Temperature Probe connections
-7	*** CAUTION *** Excess heating detected	REMOVE POWER, UNPLUG ORG and CALL ESA

For assistance in troubleshooting and replacing components, please call ESA or its authorized distributor.

14.6 Maintenance and Cleaning

The Coulochem III Organizer Modules are designed to give years of trouble free use; however, there are some general maintenance items that should be observed as with all HPLC components and systems.

⚠ CAUTION: When you are cleaning the Thermal Organizer Module, take care that the cleaning material does not get on the Temperature Probe, the Thermostat or the Heater Driver Board (located on the underside of the chassis plate) as this could damage.

In most cases, it is easier if the chassis plate and its contents are removed from the organizer tray when replacing parts. It may also be easier to service the manual injector (such as replacing tubing, connectors, etc.) with the chassis plate outside of the organizer tray.

14.6.1 Maintenance on a Daily Basis

Check all fluid connections for leaks and fix as required. Clean up any spills immediately.

14.6.2 Maintenance on a Monthly Basis

Check all cable connections including the ground strap cable. Tighten/secure as needed.

14.6.3 Cleaning

Wipe up any spilled mobile phase or other liquids immediately. The inside and outside surfaces of the organizer can be cleaned with warm soapy water and a soft cloth.

Use dilute bleach as a disinfectant. Wipe up any excess bleach and dry with a cloth.

Appendix A Coulochem® III Electrochemical Detector Specifications

General Operating Specifications

Detector Configuration:	DC potentiostat for 1 or 2 cells and Potentiostat for Guard Cell; and/or Scan Mode/Pulse Mode
Operating Modes:	DC, Pulse, Scan - depends on options installed (Timeline can be attached to DC and Pulse methods)
Potential Range:	± 2000 mV in 1 mV steps
Full Scale Output Range:	10 pA to 1 mA in 1-2-5 sequence (DC Mode)
Filter Time Constants (DC Only):	0.2 to 10 seconds in 1-2-5 sequence (DC mode)
Noise Specification:	< 750 fA peak to peak with a 500 MΩ, 0.47 µF test load and a 2 sec filter (DC Mode)
Signal Output:	± 100 mV, ± 1 V D.C.
Output Resolution DC Mode:	0.12 µV at 1 V full scale (24 bit bipolar)
Output Resolution Pulse/Scan Mode:	1.9 µV at 1 V full scale (20 bit bipolar)
Output Offset:	± 50% of the selected current range in 1% steps
Guard Cell Potential and Current:	± 2000 mV in 1 mV steps, ± 2 mA maximum current
Event Marker:	Triggered by keypad, timed operation or RS232 control, width, height, polarity and channel selectable
Autozero:	Triggered by front panel keypad, rear panel contact closure, timed operation or RS232 control
Function Keys:	Autozero, Event Marker, Cell On/Off, Run/Stop
RS232 Interface Capability:	Full parametric instrument control for DC and Pulse

Method Storage:	Up to 25 methods (any combination of DC, Scan and Pulse with Timeline)
Temperature Range: (Thermal Organizer Option)	Ambient + 5° to 60°C
Temperature Stability: (Thermal Organizer Option)	< ± 0.5°C
Warm-up Time: (Thermal Organizer Option)	< 30 minutes (typically)
Temperature Accuracy: (Thermal Organizer Option)	≤ ± 0.5°C

Scan Mode Specifications

Scan Points:	4 selectable potentials: Initial Potential, Potl Limit 1, Potl Limit 2 and Final Potential
Current Ranges:	1 nA to 10 mA in 1-2-5 step sequence
Recorder Y-axis Outputs:	± 100 mV, ± 1 V
Scan Rate:	1-1000 mV/Sec in 1 mV increments
Scan Cycle:	1 to 9999 or continuous cycle
X-Axis Divisor:	1, 2, 5, 10

Pulse Mode Specifications

Pulse Times:	Time 1: Acquisition Delay + 5 to 1000 msec Time 2: 4 to 1000 msec Time 3: 0 to 1000 msec Time 4: 0 to 1000 msec
Acquisition Delay:	50 msec to Time 1-5 msec
Pulse Voltage Range:	± 2000 mV each pulse in 1 mV increments
Charge Ranges:	10 pC to 10 mC in 1-2-5 step sequence
Recorder Outputs:	± 100 mV, ± 1 V

Timeline Specifications (DC and Pulse Mode)

Programmable Changes:	Selectable per channel at times from 0 to 9999.99 minutes in 0.01 minute increments
Programmable Events:	Current Ranges, Cell Potentials, Increment Cell Potentials, Five Output Contact Closures, Program Hold, Autozero, Event Mark, Parameter Reset, Program Loop Command, Program End Command, Filters, and Cell Off/On
Program Repeats:	1 to 9999 using Loop command
Program Start:	Via front panel keypad or rear panel contact closure
External Device Control:	Control of up to 5 external devices via contact closures

Physical Specifications

Power:	100-240 VAC, 50/60 Hz, 100 VA Max
Dimensions:	
Coulochem III:	19.5" (L) x 9" (W) x 11.25" (H) (49.5 cm x 23 cm x 28.6 cm)
Organizer Module:	18.5" (L) x 9" (W) x 5.3" (H) (47 cm x 23 cm x 13.5 cm)
Weights:	
Coulochem III:	14.8 lbs (6.7 kg)
Organizer Module:	7.6 lbs (3.4 kg)
Thermal Organizer Module:	9.1 lbs (4.0 kg)

Environmental

Operating Temperature:	10-35°C
Humidity:	Maximum 80% RH (35°C), non-condensing
Storage Temperature:	-10 to 60°C

Specifications are subject to change without notice.

Certifications

The Coulochem III Electrochemical Detector has the following Laboratory Equipment certifications:

USA:	UL 61010A-1 1st Edition
Canada:	CSA Standard C22.2 No. 1010.1-92
European Union:	EN 61326:1997 + A1:1998 EN 61010-1 (2001-02)

Appendix B Recommended Supplies and Spare Parts

B.1 Manual Injectors & Accessories

Manual Injectors & Accessories	Part Number
Model 9125-M Rheodyne Manual Injector (2 µL loop)	70-1979
Model 9125 Inert Rheodyne Injector (20 µL loop)	70-0021
10 µL Injection Syringe with 22 Gauge Blunt End Needle	50-6022
50 µL Injection Syringe with 22 Gauge Blunt End Needle	50-0872
100 µL Injection Syringe with 22 Gauge Blunt End Needle	50-6024
Needle Port Cleaner	50-0880
Syringe Needle, 22 Gauge Luer Taper	50-0883
Sample Loop, 5 µL Rheodyne, PEEK™	70-1080
Sample Loop, 10 µL Rheodyne, PEEK	70-1081
Sample Loop, 20 µL Rheodyne, PEEK	70-1082
Sample Loop, 50 µL Rheodyne, PEEK	70-1083
Sample Loop, 100 µL Rheodyne, PEEK	70-1084

B.2 Columns and Accessories

Columns and Accessories	Part Number
Column, HR-80, 4.6 mm x 8 cm	68-0100
Column, Meta-250, 4.6 mm x 25 cm with Guard Holder	70-1956
Column, Meta-250, 4.6 mm x 25 cm with Guard Holder and 4 Guard Cartridges	70-1973
Column, MCM HPLC, 5 micron, 4.6 mm x 15 cm	70-0340
Column, Microdialysis MD-150 Analytical, 3.2 mm x 15 cm	70-0636
Column, Microdialysis MD-150 x 1 Microbore, 1 mm x 15 cm	70-1745
Column, DHBA-250, 3mm x 25 cm	70-2115
Column, ACH-3 Analytical, 3.2 mm x 25 cm, with Guard Holder	70-0638
Column, ACH-3 Analytical, 3.2 mm x 25 cm, with Guard Holder and 3 Guard Cartridges	70-1042
Cartridge, Acetylcholine ACH-3-G Guard	70-0639
ACH-SPR Holder	70-1026
ACH-SPR Solid Phase Reactor for Acetylcholine	70-0640
Guard Column 20 x 4 mm, 2/pkg (used with MCM column)	70-1393
Fitting, Guard Column Holder, 2/pkg	70-1394
Cartridge, Guard C-18M 4/pkg (used with Meta-250 column)	70-1972

B.3 Filters, Pulse Dampers, Connectors, Tubing, etc.

Filters, Pulse Dampers, Connectors, Tubing, etc.	Part Number
Coulochem® Fittings & Tubing Kit	70-4604
Ferrule for Cells (Super-Flangeless™ PEEK) 5014B	70-1740
Nut, 1/16" x ¼-28 PEEK (Super-Flangeless) 5014B	70-1742
Rheodyne Long Bushing, 1/16" Male	50-0881
Rheodyne Ferrule, 1/16"	50-0882
Nut, 1/16", Valco, S.S.	50-6107
Nut, 1/8", Flangeless, Polypropylene	70-0315
In-Line Prefilter Kit, PEEK	70-0893
Filter Element, Graphite, for PEEK Prefilter (5/pk)	70-0898
Filter Element, PEEK (5/pk) (use with 70-0893)	70-3824
Tubing, Teflon®, 1/8" OD x 1/16" ID 36" long	50-0186B
Pulse Damper, PEEK	70-0894
Membrane Filters, 0.22 µm (100/pk)	40-0179
Union, 10-32, PEEK	70-1304
Syringe Filters, 0.45 µm (25/pk)	55-0807
Cell Flushing Syringe, 1/4" x 28, Threaded End	50-6020
Syringe, 30 mL Plastic, with Luer-Lok Tip	50-0873
Syringe, 1 mL Plastic, with Luer-Lok Tip	40-0190
Dispensing Tips, 15 Gauge x 1/2" (50/pk)	40-0264
Filter Assembly, 10 µm, Ultrahigh Molecular Weight Polyethylene, Solvent Reservoir	70-1302
Replacement Filter, 10 µm, Ultrahigh Molecular Weight Polyethylene	70-1303
Tubing, PEEK, 0.020" ID, Orange (specify Length)	70-0493
Tubing, PEEK, 0.010" ID, Natural (specify Length)	70-0088
Tubing, PEEK, 0.007" ID, Yellow (specify Length)	70-0492
Tubing, PEEK, 0.005" ID, Red (specify Length)	70-0491
Tubing, Cutter, for Polymeric Tubing, PEEK	70-1307
Ferrule, 1/16" Valco, S.S.	50-6105
Nut & Ferrule, 1/16" SealTight®, PEEK	70-3675
1/16" x 10-32 Knurl/Hex SealTight Nut & Ferrule (pulse damper)	70-4859
Ferrule, 1/16" Parker-CPI, S.S. (various columns)	50-6067
Nut, 1/16" Parker-CPI, S.S. (various columns)	50-6066
Ferrule, 1/16" SealTight, PEEK	70-3677
Nut & Ferrule, 1/16" SealTight, S.S. (ESA pumps)	70-3878
Nut, 1/16" Fingertight, PEEK (ESA pumps)	70-0714
Nut & Ferrule, 1/8", 5/16-24, PEEK (ESA pumps)	70-3674
Fitting, PEEK, 10-32 Plug (5014B & 5041 Cells)	70-1741
Cell Waste Line Assembly	70-1652

B.4 Mobile Phases

Mobile Phases	Part Number
Mobile Phase “A” (4 liters)	45-0171
Mobile Phase “B” (1.8 liters)	45-0168
Mobile Phase MD-TM (2 liters)	70-1332
Mobile Phase Cat-a Phase II (1 liter)	45-0216
Mobile Phase UCAT/METS (2 liters)	70-3067
Mobile Phase, Test (2 liters)	70-3829
Reagent MB Microbicide	70-1025

B.5 Cells and Electrodes

Cells and Electrodes	Part Number
Model 5010A Standard Analytical Cell, Dual Channel	55-5560
Model 5011A High Sensitivity Analytical Cell, Dual Channel	55-5561
Model 5014B Microdialysis Cell, Dual Channel	70-0520B
Model 5020 Guard Cell	55-0417
Model 5021 Conditioning Cell, Single Channel	55-0450
Model 5040 Cell with Glassy Carbon Target & Accessories	70-1075
Model 5040 Cell with Gold Target & Accessories	55-0185
Model 5040 Cell with Platinum Target & Accessories	70-1074
Model 5040 Cell with Silver Target & Accessories	70-1076
Model 5041 Cell with Glassy Carbon Target & Accessories	70-4131
Model 5041 Cell with Accessories, without Target	70-1985
Glassy Carbon/Ceramic Target for 5040/5041 Cell	70-2000
Gold/Ceramic Target for 5040/5041 Cell	70-2134
Platinum/Ceramic Target for 5040/5041 Cell	70-2135
Silver Target for 5040/5041 Cell	70-0096
Torque Wrench for 5040/5041 Cell	70-1713
Gasket, 0.005 inch (125 µm) for 5040/5041 Cell (5/pkg)	55-0188
Gasket, 0.002 inch (50 µm) for 5040/5041 Cell (5/pkg)	70-1731
Gasket, 0.001 inch (25 µm) for 5040/5041 Cell (5/pkg)	70-1730
Gasket, 0.0005 inch (12 µm) for 5040/5041 Cell (5/pkg)	70-2025
Polishing Kit, Target Electrode for 5040/5041 Cell	55-0181

B.6 Cables and Cell Simulator Loads

Cables and Cell Simulator Loads	Part Number
Dual Channel DC Cable (5010A, 5011A, 5014B Cells, DC Mode)	70-4769
Dual Channel DC Simulator Test Load (Connects to end of Dual Channel DC Cable (70-4769))	70-1790
Single Channel DC Cable (5040, 5041 Cells, DC Mode)	70-4770
Single Channel Pulse Cable (5040, 5041 Cells, Pulse Mode)	55-0179
Dual Single Channel Cable (e.g., two 5041 Cells, DC Mode)	70-5631
Single Channel DC/Pulse Simulator Test Load (Connects to end of Single Channel DC Cable (70-4770), Single Channel Pulse Cable (55-0179) and Pulse/Scan Module on CCIII)	70-4756
DC Potentiostat Cell Simulator Test Load (Connects to DC Pstat Module on CCIII)	70-4755
Guard/Conditioning Cell Cable	55-0173
Guard/Conditioning Cell Simulator Test Load	55-0172
BNC to Bare Wire Cable (Signal out to recording device)	70-1776
BNC Male to Double Binding Post Adapter	70-0219
Bare Wire to Bare Wire (I/O Connections)	70-4850
Cable RS232, Coulochem III to Computer	70-1743
Cable, Interface Thermal Organizer, 50 Watt	70-5595A

B.7 Manuals

Manuals	Part Number
Reference Manual	70-6501
User's Guide Manual	70-6502
Service Manual	70-6503

B.8 Pumps and Accessories

Pumps and Accessories	Part Number
Model 582 Solvent Delivery Module with Accessory Kit	70-4049
Mobile Phase Vacuum Degassing Unit, Non-metal, 2 Channel	70-1482
Mobile Phase Vacuum Degassing Unit, Non-metal, 3 Channel	70-1483

B.9 Autosamplers and Accessories

Autosamplers and Accessories	Part Number
Model 542 Autosampler with Sample Tray Cooling & Standard Tray	70-4151
Model 542 Autosampler with Standard Tray	70-4152
Model 540 Autosampler	70-1448
Model 540 Autosampler with Tray Temperature Control	70-1484
Model 540 Autosampler with Biocompatible Stream Switching	70-1485
Model 540 Autosampler with Tray Temperature Control and Biocompatible Stream Switching	70-1486
Vial Kit for 1.8 mL Screw Top Vials (1000/kit), includes: 1.8 mL Glass Screw Top Vial (70-1247) Plastic Cap (50-6145) Silicone/Teflon Backed Septum (50-6146)	63-0250
Vial Kit for 1.8 mL Crimp Top Vials (1000/kit), includes: 1.8 mL Glass Crimp Top Vial (70-1248) Aluminum Cap with Rubber/Teflon Coated Septum (50-6143)	63-0200
Vial Kit for 0.25 mL Plastic Crimp Top Vials (1000/kit), includes: 0.25 mL Plastic Glass Crimp Top Vial (70-1681) Aluminum Cap with Rubber/Teflon Coated Septum (50-6143)	70-1695
Vial, 1.8 mL Glass Screw Top (1000/pkg)	70-1247
Vial Cap, Plastic Screw Type for 1.8 mL Glass Screw Top Vial (1000/pkg)	50-6145
Vial Cap Septum, Silicon Teflon Backed (1000/pkg)	50-6146
Vial, 1.8 mL Glass Crimp Top (1000/pkg)	70-1248
Vial, 0.25 mL Plastic Crimp Top for Model 465 (1000/pkg)	50-6270
Vial, 0.25 mL Plastic Crimp Top (1000/pkg)	70-1681
Vial Cap, Aluminum Crimp Type with Rubber/Teflon Coated Septum (1000/pkg)	50-6143
Hand Crimper for Aluminum Caps	63-0201

B.10 Tools and Replacement Parts

Tools and Replacement Parts	Part Number
Wrench, Open End, ⅜" x 7/16"	50-0867
Wrench, Open End, ½" x 9/16"	50-0868
Wrench, Open End, 5/16" x ⅜"	50-0564
Wrench, Open End, ¼" x 5/16"	50-0366
Screwdriver, 3/32" (2" long), Slotted	50-0257
Screwdriver, #2 (4" long), Phillips	70-1049
Fuse, 1 Amp (250 V), (2 fuses are required)	70-6666

B.11 Coulochem III Accessories

Coulochem III Accessories	Part Number
Coulochem III Thermal Organizer Module	70-5499TA
Coulochem III Organizer Module	70-5499
Pulse/Scan Upgrade	70-5504
DC Upgrade	70-5505

B.12 Organizer Module Spare Parts

Organizer Module Spare Parts	Part Number
Coulochem III Organizer Accessory Kit	70-5651

B.13 Thermal Organizer Module Spare Parts

Thermal Organizer Module Spare Parts	Part Number
Coulochem III Thermal Organizer Accessory Kit	70-5651TA
Standard Column Clamp Assembly (15 cm)	70-5366
Short Column Clamp Assembly (5 cm)	70-5365
Long Column Clamp Assembly (25 cm)	70-5367
Cable, Interface Organizer to Coulochem III	70-5595

B.14 Books & Services

Books & Services	Part Number
Coulometric Electrode Array Detectors for HPLC. Progress in HPLC/HPCE, Vol.6, I.N. Acworth, M. Naoi, H. Parvez, S. Parvez	70-3419
Coulochem & HPLC System Validation	70-4741
Coulochem Detector Validation	70-4739

Appendix C Maintaining Cell Performance for Coulometric Sensors

ESA coulometric sensors (cells) for HPLC with electrochemical detection depend upon chemical reactions occurring at the electrode surface. These chemical reactions are very dependent upon pH, mobile phase, potential, and the condition of the electrode. Additionally, the condition of other components of the analytical system (pump, injector, pulse damper, etc.) can greatly affect detector performance.

Cleanliness of the water and chemicals used to prepare the mobile phase continues to be the single greatest source of problems in cell performance. 18.2 megohm-cm water that is additionally purified through a 0.22 µm filter and processed to remove trace organics, should be used for the preparation of all solutions. See ESA's Water Polishing Technical Note (Part Number 70-1668P) for detailed information.

Chemicals should be selected based on purity with low ppm (or better) of iron and other heavy metals.

C.1 Things to Do and Not To Do

C.1.1 Things to Do

- DO use an in-line pre-filter before the cell.
- DO use very clean reagents and water for mobile phase preparation.
- DO maintain the performance and cleanliness of the HPLC components.
- DO check the hydrodynamic voltammogram (HDV) of each new cell used on a Coulochem® Detector to determine its optimal potential for the desired application.
- DO turn off the potential(s) and detach the cell when chemically cleaning it or any other HPLC component.
- DO reverse the flow direction to try to reduce high cell backpressures. The Model 5014B cell must be limited to a maximum of 400 psi (28 bar).
- DO electrochemically treat the cell as outlined in the "Electrochemically Treating the Cell" section in section C.3.
- DO re-circulate the mobile phase between analyses.
- DO purge the reference electrode on Model 5014B cell before applying the potentials.
- DO make sure that there is enough mobile phase to last for the intended analysis or overnight, etc. Low flow rates or recycling of the mobile phase may be used during these times.

C.1.2 Things Not to Do

- DO NOT use nitric acid to attempt to clean a cell. While 6N nitric acid has been used to clean and passivate stainless steel-based HPLC systems (after removal of the column) for use with EC detectors, it should only be used to clean the cell as a very last resort.
- DO NOT allow mobile phase to run empty while potentials are applied to the cell.
- DO NOT allow the flow to be stopped for more than 15 minutes, while the cell has a potential(s) applied to it. Otherwise, the current will continuously increase to the point where permanent cell damage will occur.
- DO NOT allow the cell to become dry while potentials are applied to it. Always turn off the potential when working on the cell. On the Coulochem III, push the CELLS ON/OFF key.
- DO NOT operate coulometric cells above their maximum operating pressure: 5010A/5011A/5014B/5021 = 600 psi (41 bar); 5020 = 6000 psi (414 bar) and 6210 = 1200 psi (82 bar).
- DO NOT operate coulometric cells at temperatures greater than 45°C as this may damage them.
- DO NOT jump to the conclusion that a problem is a result of the cell. Often there are other explanations why the system may no longer perform as desired.

C.2 Restoring Cell Performance

When a cell does not produce the expected response or is no longer usable, a number of procedures may be tried to restore its performance.

☞ NOTE: If an assay is critical, it is wise to have a replacement cell available before trying these procedures.

The suggestions on the following pages have been found to restore cell performance. However, in some situations it is possible that performance will not improve and may even be made worse.

The apparent loss of response (decrease in peak height and area) in the cell can be a result of many factors. Changes in the HPLC components, degradation of standards in solution and auto-oxidation of the sample on column should be eliminated.

If the effect is isolated to the cell, the loss of response could be due to:

- Poisoning or coating of the internal electrode(s)
- Shift in the HDV
- Age of the cell
- Physical damage to the cell
- Clogging of the porous graphite electrode(s)

NOTE: In many cases the first two effects occur simultaneously.

C.3 Electrochemically Treating the Cell

a) Turn off the potential(s) to the cell(s).

b) Replace the mobile phase with fresh phase (the mobile phase should be of low organic solvent composition, i.e., less than 15%) flowing at about 1 mL/minute.

c) Apply a potential of +1000 mV to the electrode(s) for 10 minutes with mobile phase flowing. **Do not recycle the mobile phase** during the treatment. The currents will more likely over-range, this is normal.

d) Reset the potentials to the assay's working potentials.

e) Establish a stable baseline and test the response.

f) If the response has not returned to the expected level reset the potential to a negative value -350 to -450 mV for 10 minutes.

g) Reset the potential and again test under operating conditions.

If response is not back to normal at this point, a combination of potentials should be tried:

a) With fresh mobile phase flowing, set the potential to -350 to -450 mV for 10 minutes. Follow this treatment by setting the potentials to +1000 mV for 30 minutes.

b) Return to operating conditions. Once a stable baseline is achieved, evaluate the response.

If these procedures fail, the cell may still be used by changing to different potentials. The new potentials can be determined by the performance of an HDV (see Operating Manual).

☞ NOTE: Success with this procedure is very dependent on the mobile phase used, its cleanliness and the operating conditions. Under some conditions this procedure may provide only minimal improvement in response or even make the cell's performance worse.

C.4 Sharpening the HDV - Series 5000 Cells Only

The variation observed in the HDV from one cell to another can be minimized by a simple electrochemical pretreatment of the electrode surface.

When using the cell for analysis, response can often be restored by shifting the HDV curve back to its initial position. (A shift in HDV can be verified by initially setting the potential to +300 mV higher than normally used. If the response is restored, the problem is probably a shift in HDV.)

The procedure is:

a) Replace the mobile phase with ESA's MC-TM mobile phase (Part Number 70-1332) flowing at 1.0 mL/minute. **Do not recycle.**

b) After allowing the mobile phase to flow for 1 minute, set the electrode potentials to +1000 mV for 30 minutes.

c) Replace the MC-TM mobile phase with your mobile phase. Reset the potentials to analysis conditions and allow the baseline to stabilize. This may take several hours, depending on the sensitivity range required.

d) Evaluate the response with the appropriate standards. (It is recommended that the standards be freshly prepared to eliminate degradation of the standard as a possible cause of the problem.)

If the response has not been restored, this procedure may be repeated one more time. If the response is not restored after the second treatment, the cell may need to be replaced. We do not recommend that this procedure be done routinely, but rather only when a shift in the HDV is observed.

C.5 High Backpressure

Occasionally a cell will exhibit normal performance but have unusually high backpressure. The cell may continue to be used even with the increased backpressure if it exhibits normal analytical behavior. The backpressure may be reduced with this procedure:

a) Turn off the cell potential and stop the flow.

⚠ CAUTION: Always turns off the pump and let the system pressure drop slowly to zero before disconnecting any components on the high-pressure side. Failure to do so can ruin the column and rupture a pulse damper membrane, which could then lead to noise, high background current and high backpressure.

b) Remove the cell from the system.

c) Prepare a 60% phosphoric acid solution by measuring out 200 mL of pure water and adding 500 mL of 85% phosphoric acid.

⚠ WARNING: Wear protective eyewear, laboratory coat, and gloves when handling phosphoric acid. Dispose of used material appropriately.

d) Using a pump or glass syringe (Part Number 50-6020) connected to the cell outlet, first flush with water (at least 3 mL) followed by the phosphoric acid solution. Follow the phosphoric acid solution with pure water (at least 3 mL).

☞ NOTE: Do not allow the phosphoric acid to reside inside the cell for longer than 1 minute.

C.6 Special Procedures

In some cases the cause of the decrease in cell performance is known and is due to the analytical method. In these cases, the ultimate solution is to modify the method so that cell life is extended. In some cases, however, the cell response may be restored by one of the following procedures.

C.7 Lipophilic Materials

Lipophilic materials typically come from the sample, contaminated buffers (bacteria), or water.

⚠ CAUTION: In most cases the lipophilic material will also be on the column. Therefore, the column should be washed separately (disconnected) prior to cleaning the cells, using this same procedure. Check the column manufacturer's care and use literature to verify compatibility with these solvents.

a) Turn off the cell potential(s) and stop mobile phase flow.

b) Remove the column and cell(s) from the system.

c) Remove mobile phase from the cell by flushing with pure water for 10 minutes at 1 mL/minute.

d) Sequentially flush with methanol, acetonitrile, THF (tetrahydrofuran), acetonitrile, methanol, and back to pure water all at a rate of 1 mL/minute for 10 to 15 minutes each.

e) Change the pre-filter element directly in front of the cell.

f) Return to mobile phase, set potentials, equilibrate column and attach the cell(s), then test the response.

C.8 Silica in the Cell

Silica fines from the column or guard column may reach the cell and cause high backpressure or unusual performance.

☞ NOTE: Use an in-line pre-filter after the column to prevent this occurrence.

The silica can be removed as follows:

a) Turn off the cell potentials(s) and flush the system with pure water for 10 to 15 minutes at 1 mL/minute.

b) Remove the column from the system.

NOTE: This procedure will destroy a silica-based column if it is left in place.

c) Prepare a 2 M sodium hydroxide solution.

WARNING: Wear protective eyewear, laboratory coat, and gloves when handling. Dispose of used material appropriately.

d) Using a syringe, fill the cell with the 2 M sodium hydroxide. Leave the solution in the cell for 10 to 30 minutes.

e) Flush the cell with 10 mL of water.

f) Re-attach the cell to the system and pump pure water until the eluent is at neutral pH.

g) Replace the column and mobile phases, set the potentials, equilibrate and test the system.

C.9 Hints for Improved Performance

A very "clean", "quiet", and well-maintained chromatographic system is vital to performance. Failure to provide a clean system (i.e., mobile phase and system components) can lead to high background currents, high noise, and low signal. In many cases the use of PEEK, Teflon®, Tefzel® and other inert polymer-based HPLC components can lead to better performance than those made of stainless steel. This will ultimately depend on the conditions used and the level of sensitivity required for the analyses.

Good, well-maintained HPLC components are necessary for analyzing low analyte levels as these can affect the noise level of the baseline. The use of a quiet pump with proper pulse control (dampers) can greatly minimize the noise of the baseline.

The ESA HPLC pumps and pulse dampers have been specifically configured to minimize pump pulsations and eliminate materials that could contaminate the mobile phase.

Other HPLC components such as tubing, injectors, columns, filters, zero dead volume connectors, etc., must also be of high quality and be well maintained in order to consistently provide low-level detection.

ESA offers a full range of HPLC components that have been selected to meet the demanding requirements for low level EC detection.

Check to make sure that there are no leaks in the system particularly at connections, as leaks may lead to baseline artifacts and noise. At low flow rates, leaks can be difficult to detect. Look for salt crystallization at joints, etc.

A malfunctioning pump, pulse damper, or injector could cause increased noise, high background currents, high backpressure and interfering peaks.

Noise may also be minimized by making sure that the end of the outlet waste tubing is under the surface of the waste mobile phase and not "dripping" into it. Drips can cause minor pressure variations that can be picked up by the cell and seen as noise.

Make sure that all of the metal HPLC components are well grounded to the same ground. If not, "ground loops" may form which can lead to increase baseline noise and other baseline artifacts. An electrician may need to be consulted to check on the condition of your laboratory's electrical grounds. If using a Coulochem Organizer, makes sure it is grounded to Coulochem Detector. Grounding various components of the HPLC system (for example pump heads, pulse dampers, stainless steel tubing, columns, etc.) may result in reduced baseline noise.

Typically the entire analytical system is first plugged into a power strip and then plugged into the power outlet or UPS system.

The use of a thermostated chamber (45°C max.) for most of the HPLC components (injector, column and cell) may provide several advantages. Holding the column (and to a lesser extent the injector) at a constant temperature will provide more constant retention times from analysis to analysis.

The retention time can vary significantly with typical "normal" laboratory temperature changes and the variation can make identification and quantification of peaks more difficult. Thermostating the cell may provide a reduction in baseline noise.

The expected life of a cell is highly dependent on the potentials used, mobile phase, care and maintenance, and sample matrix. A cell that is not usable for one assay may still be usable for another.

After the appropriate procedure(s) has been tried and if cell performance has not been restored or improved to a usable level, replace the cell.

Appendix D Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET

CATAPHASE® MOBILE PHASE

SECTION A - IDENTITY

Manufacturer's Name:	ESA, Inc.
Address:	22 Alpha Road, Chelmsford, MA 01824-4171
Telephone Number:	(978) 250-7000
Trade Name and Synonyms:	Cataphase; HPLC Mobile Phase
Chemical Family:	Acid solution
CAS #:	N/A

SECTION B - HAZARDOUS INGREDIENTS

		%
Methanol	CAS # 00067-56-1	3%
Phosphoric Acid	CAS # 7664-38-2	<1%

SECTION C - PHYSICAL DATA

Boiling Point:	N/D	Specific Gravity:	N/D
Vapor Pressure (mm Hg):	N/D	Percent Volatile by Volume (%):	N/D
Vapor Density (air=1):	N/D	Evaporation Rate:	N/D
Solubility in Water:	Complete	Reactivity in Water:	None
Appearance and Odor:	Clear, slight odor		

SECTION D - FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used):	>100°C (Closed cup)
Flammable Limits:	N/D
Extinguishing Media:	Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam.

Special Fire Fighting Procedures:

None.

Unusual Fire and Explosion Hazards:

None.

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SECTION E - HEALTH HAZARD DATA

OSHA Permissible Exposure Limit:	200 ppm as Methyl Alcohol
ACGIH Threshold Limit Value:	200 ppm as Methyl Alcohol

Effects of Overexposure:

Acute Effects: Vapors may be irritating to skin, eyes nose and throat.
Chronic Effects: Chronic irritation of exposed tissues.

Emergency and First Aid Procedures:

Inhalation:	Remove to fresh air. Support breathing if necessary. Seek medical attention if irritation persists.
Eyes:	Flush eyes with water for 15 minutes. If irritation persists, see a physician.
Skin:	Wash with plenty of water. Remove saturated clothing. Seek medical attention if irritation persists.
Ingestion:	If swallowed, wash out mouth with water and see a physician.

SECTION F - REACTIVITY DATA

Avoid heat, sources of ignition, and flame. Incompatible with strong oxidizing agents.

SECTION G - SPILL OR LEAK PROCEDURES

Steps to be Taken in Case of Spill:

Pick up with sand or other non-combustible absorbent material and place in container for hazardous disposal.

Waste Disposal Method:

Dispose of according to applicable federal, state, and local regulations.

SECTION H - SPECIAL PROTECTION INFORMATION

Use general or local exhaust ventilation to meet TLV requirements. Respiratory protection required if airborne concentration exceeds TLV. Safety goggles, face shield rubber gloves and protective suit are recommended.

SECTION I - SPECIAL PRECAUTIONS

Precautions to be Taken in Handling and Storing:

Keep container tightly closed. Store in cool, dry, well ventilated storage area out of direct sunlight.

James Mayol, QA Manager

While the information and recommendations set forth herein are believed to be accurate as of the date hereof, ESA, Inc. makes no warranty with respect thereto and disclaims all liability from reliance thereon.

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Rev. A

MATERIAL SAFETY DATA SHEET

MD-TM MOBILE PHASE

SECTION A - IDENTITY

Manufacturer's Name:	ESA, Inc.
Address:	22 Alpha Road, Chelmsford, MA 01824-4171
Telephone Number:	(978) 250-7000
Trade Name and Synonyms:	MD-TM Mobile Phase
Chemical Family:	Buffered acidic solution
CAS #:	N/A

SECTION B - HAZARDOUS INGREDIENTS

		%
Acetonitrile	CAS # 75-05-8	10%
Phosphoric Acid	CAS # 7664-38-2	<1%

SECTION C - PHYSICAL DATA

Boiling Point:	N/D	Specific Gravity:	N/D
Vapor Pressure (mm Hg):	N/D	Percent Volatile by Volume (%):	N/D
Vapor Density (air=1):	N/D	Evaporation Rate:	N/D
Solubility in Water:	Complete	Reactivity in Water:	None
Appearance and Odor:	Colorless liquid with a characteristic, sweet odor		

SECTION D - FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used):	30°C (Closed cup)
Flammable Limits:	N/D
Extinguishing Media:	Carbon dioxide, dry chemical powder, alcohol or polymer foam.

Special Fire Fighting Procedures:

Use carbon dioxide, alcohol foam, or dry chemical. Wear self contained breathing apparatus and protective clothing.

Unusual Fire and Explosion Hazards:

Closed containers exposed to heat may explode. Contact with strong oxidizers may cause fire.

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Rev. A

SECTION E - HEALTH HAZARD DATA

OSHA Permissible Exposure Limit:	40 ppm ceiling as acetonitrile
ACGIH Threshold Limit Value:	40 ppm ceiling as acetonitrile

Effects of Overexposure:

Acute Effects:	Vapors may be irritating to skin, eyes nose and throat.
Chronic Effects:	Chronic irritation of exposed tissues.

Emergency and First Aid Procedures:

Inhalation:	Remove to fresh air. Support breathing if necessary. Seek medical attention if irritation persists.
Eyes:	Flush eyes with water for prolonged period. If irritation persists, see a physician.
Skin:	Wash with plenty of water. Remove saturated clothing. Seek medical attention if irritation persists.
Ingestion:	If ingested, immediately induce vomiting. See a physician immediately.

SECTION F - REACTIVITY DATA

Avoid heat, sources of ignition, and flame. Incompatible with strong oxidizing agents.

SECTION G - SPILL OR LEAK PROCEDURES

Steps to be Taken in Case of Spill:

Shut off all ignition sources. Cover with an activated carbon or other non-combustible absorbent material and place in container for hazardous disposal. Ventilate area and wash spill site after material pickup is complete.

Waste Disposal Method:

Dispose of according to applicable federal and state regulations.

SECTION H - SPECIAL PROTECTION INFORMATION

NIOSH/OSHA respiratory protection should be used. Impervious material gloves and heavy boots should be worn. Safety glasses with side shields or goggles should be worn. Use only in well ventilated area.

Emergency shower and eye wash stations should be within work area. Avoid contact with product and wash thoroughly after each handling.

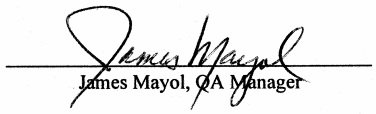
SECTION I - SPECIAL PRECAUTIONS

Precautions to be Taken in Handling and Storing:

Keep container tightly closed. Store in cool, dry, well ventilated storage area out of direct sunlight.

Other Precautions:

Do not get in eyes, on clothing or skin.



James Mayol, QA Manager

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MATERIAL SAFETY DATA SHEET

MOBILE PHASE A

SECTION A - IDENTITY

Manufacturer's Name:	ESA, Inc.
Address:	22 Alpha Road, Chelmsford, MA 01824-4171
Telephone Number:	(978) 250-7000
Trade Name and Synonyms:	Mobile Phase A; HPLC Mobile Phase
Chemical Family:	Phosphate Buffer Solution
CAS #:	N/A

SECTION B - HAZARDOUS INGREDIENTS

Mobile Phase A is not considered a hazardous product. ESA, Inc. is not aware of any hazards associated with this product.

James Mayol, QA Manager

While the information and recommendations set forth herein are believed to be accurate as of the date hereof, ESA, Inc. makes no warranty with respect thereto and disclaims all liability from reliance thereon.

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Rev. A

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MATERIAL SAFETY DATA SHEET

MOBILE PHASE B

SECTION A - IDENTITY

Manufacturer's Name:	ESA, Inc.
Address:	22 Alpha Road, Chelmsford, MA 01824-4171
Telephone Number:	(978) 250-7000
Trade Name and Synonyms:	Mobile Phase B
Chemical Family:	Buffered acidic solution
CAS #:	N/A

SECTION B - HAZARDOUS INGREDIENTS

		%
Methanol	CAS # 00067-56-1	50%
Phosphoric Acid	CAS # 7664-38-2	<1%

SECTION C - PHYSICAL DATA

Boiling Point:	N/D	Specific Gravity:	N/D
Vapor Pressure (mm Hg):	N/D	Percent Volatile by Volume (%):	50% methanol
Vapor Density (air=1):	N/D	Evaporation Rate:	N/D
Solubility in Water:	Complete	Reactivity in Water:	None
Appearance and Odor:	Colorless liquid with a characteristic, pungent odor		

SECTION D - FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used):	N/D
Flammable Limits:	N/D
Extinguishing Media:	Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam.

Special Fire Fighting Procedures:

Use water spray, alcohol foam, or dry chemical. Flush spill area with water.

Unusual Fire and Explosion Hazards:

Closed containers exposed to heat may explode. Contact with strong oxidizers may cause fire.

MSDS-013
Rev. A

SECTION E - HEALTH HAZARD DATA

OSHA Permissible Exposure Limit:	200 ppm
ACGIH Threshold Limit Value:	200 ppm

Effects of Overexposure:

Acute Effects:	Vapors may be irritating to skin, eyes nose and throat.
Chronic Effects:	Chronic irritation of exposed tissues.

Emergency and First Aid Procedures:

Inhalation:	Remove to fresh air. Support breathing if necessary. Seek medical attention if irritation persists.
Eyes:	Flush eyes with water for 15 minutes. If irritation persists, see a physician.
Skin:	Wash with plenty of water. Remove saturated clothing. Seek medical attention if irritation persists.
Ingestion:	If ingested, immediately induce vomiting. See a physician immediately.

SECTION F - REACTIVITY DATA

Avoid heat, sources of ignition, and flame. Incompatible with strong oxidizing agents.

SECTION G - SPILL OR LEAK PROCEDURES

Steps to be Taken in Case of Spill:

Shut off ignition sources. Stop leak if you can without risk. Use water spray to reduce vapors. Pick up with sand or other non-combustible absorbent material and place in container for hazardous disposal. Flush area with water.

Waste Disposal Method:

Dispose of according to applicable federal and state regulations.

SECTION H - SPECIAL PROTECTION INFORMATION

Use general or local exhaust ventilation to meet TLV requirements. Respiratory protection required if airborne concentration exceeds TLV. Safety goggles, face shield rubber gloves and protective suit are recommended.

SECTION I - SPECIAL PRECAUTIONS

Precautions to be Taken in Handling and Storing:

Keep container tightly closed. Store in cool, dry, well ventilated storage area out of direct sunlight.

Other Precautions:

Do not get in eyes, on clothing or skin.

James Mayol, QA Manager

While the information and recommendations set forth herein are believed to be accurate as of the date hereof, ESA, Inc. makes no warranty with respect thereto and disclaims all liability from reliance thereon.

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Rev. A

MATERIAL SAFETY DATA SHEET

REAGENT MB MICROBICIDE

SECTION A - IDENTITY

Manufacturer's Name:	ESA, Inc.
Address:	22 Alpha Road, Chelmsford, MA 01824-4171
Telephone Number:	(978) 250-7000
Trade Name and Synonyms:	MB Reagent
Chemical Family:	Microbicide
CAS #:	N/A

SECTION B - HAZARDOUS INGREDIENTS

Distilled Water	CAS #7732-18-5	75.75%
Magnesium Nitrate	CAS #10377-60-3	22.00%
5-chloro-2-methyl-4-isothiazolin-3-one	CAS #26172-55-4	1.15%
2-methyl-4-isothiazolin-3-one	CAS #2682-20-4	0.35%
Magnesium Chloride	CAS #7786-30-3	0.75%

SECTION C - PHYSICAL DATA

Boiling Point:	100°C	Specific Gravity:	1.2 G/ml
Vapor Pressure (mm Hg):	21 mm	Percent Volatile by Volume:	75%
Vapor Density (air = 1):	0.65	Evaporation Rate:	<1.0
Solubility in Water:	Complete	Melting Point:	-21°C
Appearance and Odor:	Amber liquid, pungent odor		

SECTION D - FIRE AND EXPLOSION DATA

Flash Point (Method Used):	N/A
Flammable Limits:	Lel: N/A, Uel: N/A
Extinguishing Media:	This material is not flammable.

Special Fire Fighting Procedures:

Wear self-contained breathing apparatus when fighting a chemical fire.

Unusual Fire and Explosion Hazards:

The following toxic vapors are found when this material is heated to decomposition: oxides of nitrogen and sulfur, hydrogen chloride.

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Rev.A

SECTION E - HEALTH HAZARD DATA

OSHA Permissible Exposure Limit:	0.3 mg/m3 as total isothiazolones
ACGIH Threshold Limit Value:	0.1 mg/m3 as total isothiazolones

Effects of Overexposure:

Burns eyes severely, dermatitis, burns skin, system allergic reactions.

Emergency and First Aid Procedures:

Inhalation:	Immediately move to fresh air.
Eyes:	Flush eyes with water for 15 minutes.
Skin:	Promptly wash skin with mild soap and large volumes of water.
Ingestion:	Never give anything by mouth to an unconscious person. Never try to make the unconscious person vomit. Give large amounts of water. Contact a physician.

SECTION F - REACTIVITY DATA

Stability Stable. Hazardous decomposition products: oxides of nitrogen and sulfur, hydrogen chloride. Hazardous polymerization will not occur.

SECTION G - SPILL OR LEAK PROCEDURES

Steps to be taken in case of spill:

Clean up with absorbent material.

Waste disposal method:

Dispose of according to federal and state regulations.

SECTION H - SPECIAL PROTECTION INFORMATION

NIOSH/OSHA respiratory protection should be used. Impervious material gloves should be worn. Safety glasses with side shields or goggles should be worn. Use only in well ventilated area.

Emergency shower and eye wash stations should be within work area. Avoid contact with product and wash thoroughly after handling.

SECTION I - SPECIAL PRECAUTIONS

Precautions to be taken in handling and storing:

Store in sealed container in a cool, dry location.

Other Precautions:

Avoid eye or skin contact. Avoid breathing vapors.

James Mayol, QA Manager

While the information and recommendations set forth herein are believed to be accurate as of the date hereof, ESA, Inc. makes no warranty with respect thereto and disclaims all liability from reliance thereon.

MSDS-018
Rev.A

MATERIAL SAFETY DATA SHEET

CATAPHASE II® MOBILE PHASE

SECTION A - IDENTITY

Manufacturer's Name:	ESA, Inc.
Address:	22 Alpha Road, Chelmsford, MA 01824-4171
Telephone Number:	(978) 250-7000
Trade Name and Synonyms:	Cataphase II; HPLC Mobile Phase
Chemical Family:	Acid solution
CAS #:	N/A

SECTION B - HAZARDOUS INGREDIENTS

		%
Methanol	CAS # 00067-56-1	16%
Acetonitrile	CAS # 75-05-8	8%
Phosphoric Acid	CAS # 7664-38-2	<1%

SECTION C - PHYSICAL DATA

Boiling Point:	N/D	Specific Gravity:	N/D
Vapor Pressure (mm Hg):	N/D	Percent Volatile by Volume (%):	N/D
Vapor Density (air=1):	N/D	Evaporation Rate:	N/D
Solubility in Water:	Complete	Reactivity in Water:	None
Appearance and Odor:	Clear, slight odor		

SECTION D - FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used):	30°C (Closed cup)
Flammable Limits:	N/D
Extinguishing Media:	Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam.

Special Fire Fighting Procedures:

None.

Unusual Fire and Explosion Hazards:

None.

MSDS-027
Rev. A

SECTION E - HEALTH HAZARD DATA

OSHA Permissible Exposure Limit:	200 ppm as Methyl Alcohol / 40 ppm as Acetonitrile
ACGIH Threshold Limit Value:	200 ppm as Methyl Alcohol / 40 ppm as Acetonitrile

Effects of Overexposure:

Acute Effects:	Vapors may be irritating to skin, eyes nose and throat.
Chronic Effects:	Chronic irritation of exposed tissues.

Emergency and First Aid Procedures:

Inhalation:	Remove to fresh air. Support breathing if necessary. Seek medical attention if irritation persists.
Eyes:	Flush eyes with water for 15 minutes. If irritation persists, see a physician.
Skin:	Wash with plenty of water. Remove saturated clothing. Seek medical attention if irritation persists.
Ingestion:	If swallowed, wash out mouth with water and see a physician.

SECTION F - REACTIVITY DATA

Avoid heat, sources of ignition, and flame. Incompatible with strong oxidizing agents.

SECTION G - SPILL OR LEAK PROCEDURES

Steps to be Taken in Case of Spill:

Pick up with sand or other non-combustible absorbent material and place in container for hazardous disposal.

Waste Disposal Method:

Dispose of according to applicable federal, state, and local regulations.

SECTION H - SPECIAL PROTECTION INFORMATION

Use general or local exhaust ventilation to meet TLV requirements. Respiratory protection required if airborne concentration exceeds TLV. Safety goggles, face shield rubber gloves and protective suit are recommended.

SECTION I - SPECIAL PRECAUTIONS

Precautions to be Taken in Handling and Storing:

Keep container tightly closed. Store in cool, dry, well ventilated storage area out of direct sunlight.

James Mayol, QA Manager

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MSDS-027
Rev. A

MATERIAL SAFETY DATA SHEET

MOBILE PHASE TEST

SECTION A - IDENTITY

Manufacturer's Name:	ESA, Inc.
Address:	22 Alpha Road, Chelmsford, MA 01824-4171
Telephone Number:	(978) 250-7000
Trade Name and Synonyms:	Mobile Phase Test
Chemical Family:	Buffered acidic solution
CAS #:	N/A

SECTION B - HAZARDOUS INGREDIENTS

		%
Acetonitrile	CAS # 75-05-8	10%
Phosphoric Acid	CAS # 7664-38-2	<1%

SECTION C - PHYSICAL DATA

Boiling Point:	N/D	Specific Gravity:	N/D
Vapor Pressure (mm Hg):	N/D	Percent Volatile by Volume (%):	N/D
Vapor Density (air=1):	N/D	Evaporation Rate:	N/D
Solubility in Water:	Complete	Reactivity in Water:	None
Appearance and Odor:	Colorless liquid with a characteristic, sweet odor		

SECTION D - FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used):	30°C (Closed cup)
Flammable Limits:	N/D
Extinguishing Media:	Carbon dioxide, dry chemical powder, alcohol or polymer foam.

Special Fire Fighting Procedures:

Use carbon dioxide, alcohol foam, or dry chemical. Wear self contained breathing apparatus and protective clothing.

Unusual Fire and Explosion Hazards:

Closed containers exposed to heat may explode. Contact with strong oxidizers may cause fire.

MSDS-034
Rev. B

SECTION E - HEALTH HAZARD DATA

OSHA Permissible Exposure Limit:	40 ppm ceiling as acetonitrile
ACGIH Threshold Limit Value:	40 ppm ceiling as acetonitrile

Effects of Overexposure:

Acute Effects:	Vapors may be irritating to skin, eyes nose and throat.
Chronic Effects:	Chronic irritation of exposed tissues.

Emergency and First Aid Procedures:

Inhalation:	Remove to fresh air. Support breathing if necessary. Seek medical attention if irritation persists.
Eyes:	Flush eyes with water for prolonged period. If irritation persists, see a physician.
Skin:	Wash with plenty of water. Remove saturated clothing. Seek medical attention if irritation persists.
Ingestion:	If ingested, immediately induce vomiting. See a physician immediately.

SECTION F - REACTIVITY DATA

Avoid heat, sources of ignition, and flame. Incompatible with strong oxidizing agents.

SECTION G - SPILL OR LEAK PROCEDURES

Steps to be Taken in Case of Spill:

Shut off all ignition sources. Cover with an activated carbon or other non-combustible absorbent material and place in container for hazardous disposal. Ventilate area and wash spill site after material pickup is complete.

Waste Disposal Method:

Dispose of according to applicable federal and state regulations.

SECTION H - SPECIAL PROTECTION INFORMATION

NIOSH/OSHA respiratory protection should be used. Impervious material gloves and heavy boots should be worn. Safety glasses with side shields or goggles should be worn. Use only in well ventilated area.

Emergency shower and eye wash stations should be within work area. Avoid contact with product and wash thoroughly after each handling.

SECTION I - SPECIAL PRECAUTIONS

Precautions to be Taken in Handling and Storing:

Keep container tightly closed. Store in cool, dry, well ventilated storage area out of direct sunlight.

Other Precautions:

Do not get in eyes, on clothing or skin.

Harold Asp, QA Manager

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MSDS-034
Rev. B

MATERIAL SAFETY DATA SHEET

ACID METABOLITES PHASE A

SECTION A - IDENTITY

Manufacturer's Name:	ESA, Inc.
Address:	22 Alpha Road, Chelmsford, MA 01824-4171
Telephone Number:	(978) 250-7000
Trade Name and Synonyms:	Acid Metabolites Phase A
Chemical Family:	Acid solution
CAS #:	N/A

SECTION B - HAZARDOUS INGREDIENTS

		%
Methanol	CAS # 00067-56-1	1%
Phosphoric Acid	CAS # 7664-38-2	Trace

SECTION C - PHYSICAL DATA

Boiling Point:	N/D	Specific Gravity:	N/D
Vapor Pressure (mm Hg):	N/D	Percent Volatile by Volume (%):	N/D
Vapor Density (air=1):	N/D	Evaporation Rate:	N/D
Solubility in Water:	Complete	Reactivity in Water:	None
Appearance and Odor:	Clear, slight odor		

SECTION D - FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used):	>100°C (Closed cup)
Flammable Limits:	N/D
Extinguishing Media:	Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam.

Special Fire Fighting Procedures:

None.

Unusual Fire and Explosion Hazards:

None.

MSDS-040
Rev. A

SECTION E - HEALTH HAZARD DATA

OSHA Permissible Exposure Limit:	200 ppm as Methyl Alcohol
ACGIH Threshold Limit Value:	200 ppm as Methyl Alcohol

Effects of Overexposure:

Acute Effects: Vapors may be irritating to skin, eyes nose and throat.
Chronic Effects: Chronic irritation of exposed tissues.

Emergency and First Aid Procedures:

Inhalation:	Remove to fresh air. Support breathing if necessary. Seek medical attention if irritation persists.
Eyes:	Flush eyes with water for 15 minutes. If irritation persists, see a physician.
Skin:	Wash with plenty of water. Remove saturated clothing. Seek medical attention if irritation persists.
Ingestion:	If swallowed, wash out mouth with water and see a physician.

SECTION F - REACTIVITY DATA

Avoid heat, sources of ignition, and flame. Incompatible with strong oxidizing agents.

SECTION G - SPILL OR LEAK PROCEDURES

Steps to be Taken in Case of Spill:

Pick up with sand or other non-combustible absorbent material and place in container for hazardous disposal.

Waste Disposal Method:

Dispose of according to applicable federal, state, and local regulations.

SECTION H - SPECIAL PROTECTION INFORMATION

Use general or local exhaust ventilation to meet TLV requirements. Respiratory protection required if airborne concentration exceeds TLV. Safety goggles, face shield rubber gloves and protective suit are recommended.

SECTION I - SPECIAL PRECAUTIONS

Precautions to be Taken in Handling and Storing:

Keep container tightly closed. Store in cool, dry, well ventilated storage area out of direct sunlight.

Harold Asp 10/27/00
Harold Asp, QA Manager

While the information and recommendations set forth herein are believed to be accurate as of the date hereof, ESA, Inc. makes no warranty with respect thereto and disclaims all liability from reliance thereon.

MSDS-040
Rev. A

MATERIAL SAFETY DATA SHEET

ACID METABOLITES PHASE B

SECTION A - IDENTITY

Manufacturer's Name:	ESA, Inc.
Address:	22 Alpha Road, Chelmsford, MA 01824-4171
Telephone Number:	(978) 250-7000
Trade Name and Synonyms:	Acid Metabolites Phase B
Chemical Family:	Buffered acidic solution
CAS #:	N/A

SECTION B - HAZARDOUS INGREDIENTS

		%
Methanol	CAS # 00067-56-1	50%
Phosphoric Acid	CAS # 7664-38-2	<1%

SECTION C - PHYSICAL DATA

Boiling Point:	N/D	Specific Gravity:	N/D
Vapor Pressure (mm Hg):	N/D	Percent Volatile by Volume (%):	50% methanol
Vapor Density (air=1):	N/D	Evaporation Rate:	N/D
Solubility in Water:	Complete	Reactivity in Water:	None
Appearance and Odor:	Colorless liquid with a characteristic, pungent odor		

SECTION D - FIRE AND EXPLOSION HAZARD DATA

Flash Point (Method Used):	N/D
Flammable Limits:	N/D
Extinguishing Media:	Water spray, carbon dioxide, dry chemical powder, alcohol or polymer foam.

Special Fire Fighting Procedures:

Use water spray, alcohol foam, or dry chemical. Flush spill area with water.

Unusual Fire and Explosion Hazards:

Closed containers exposed to heat may explode. Contact with strong oxidizers may cause fire.

MSDS-041
Rev. A

SECTION E - HEALTH HAZARD DATA

OSHA Permissible Exposure Limit:	200 ppm
ACGIH Threshold Limit Value:	200 ppm

Effects of Overexposure:

Acute Effects: Vapors may be irritating to skin, eyes nose and throat.
Chronic Effects: Chronic irritation of exposed tissues.

Emergency and First Aid Procedures:

Inhalation:	Remove to fresh air. Support breathing if necessary. Seek medical attention if irritation persists.
Eyes:	Flush eyes with water for 15 minutes. If irritation persists, see a physician.
Skin:	Wash with plenty of water. Remove saturated clothing. Seek medical attention if irritation persists.
Ingestion:	If ingested, immediately induce vomiting. See a physician immediately.

SECTION F - REACTIVITY DATA

Avoid heat, sources of ignition, and flame. Incompatible with strong oxidizing agents.

SECTION G - SPILL OR LEAK PROCEDURES

Steps to be Taken in Case of Spill:

Shut off ignition sources. Stop leak if you can without risk. Use water spray to reduce vapors. Pick up with sand or other non-combustible absorbent material and place in container for hazardous disposal. Flush area with water.

Waste Disposal Method:

Dispose of according to applicable federal and state regulations.

SECTION H - SPECIAL PROTECTION INFORMATION

Use general or local exhaust ventilation to meet TLV requirements. Respiratory protection required if airborne concentration exceeds TLV. Safety goggles, face shield rubber gloves and protective suit are recommended.

SECTION I - SPECIAL PRECAUTIONS

Precautions to be Taken in Handling and Storing:

Keep container tightly closed. Store in cool, dry, well ventilated storage area out of direct sunlight.

Other Precautions:

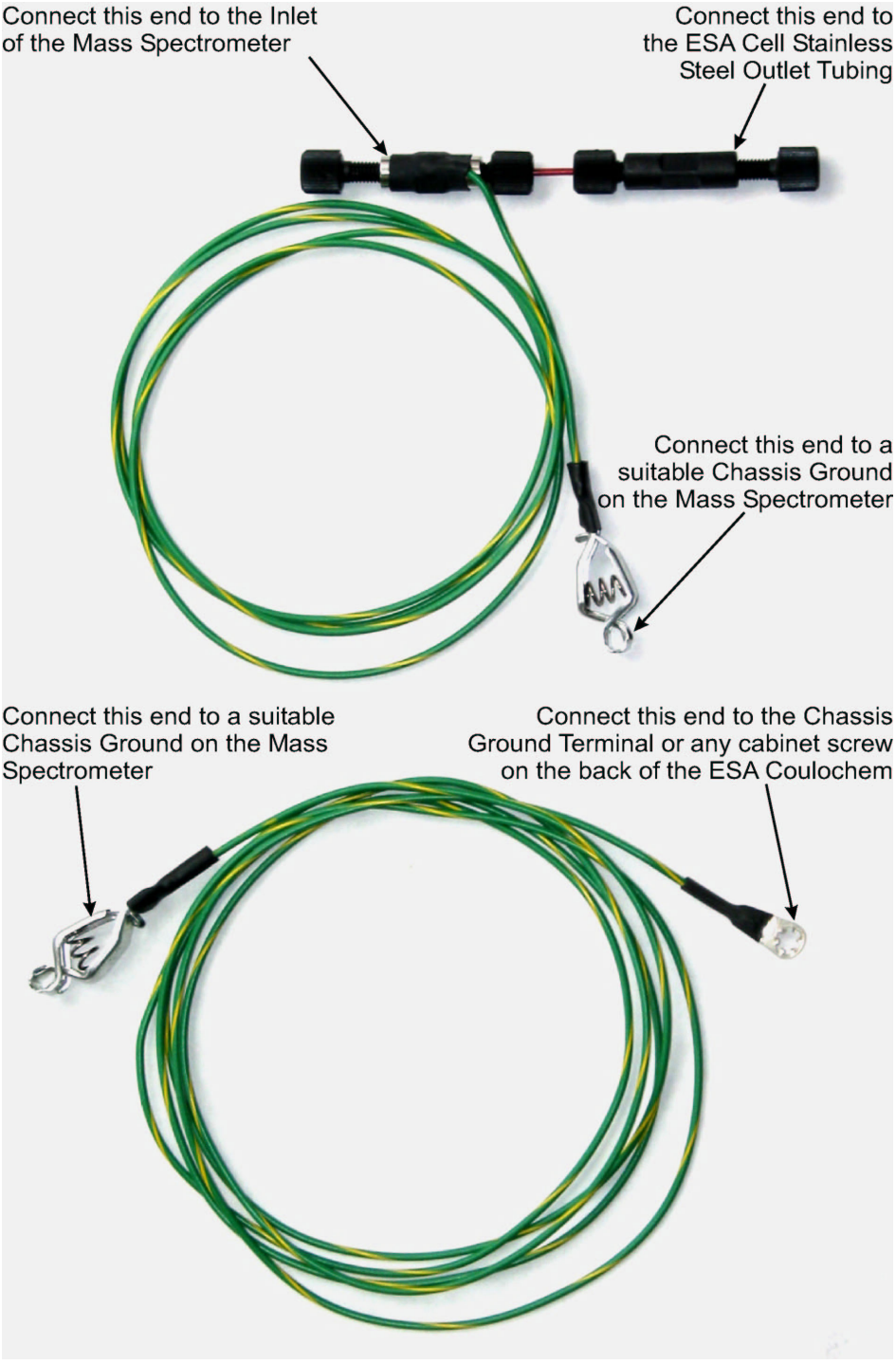
Do not get in eyes, on clothing or skin.

Harold Asp 10/22/00
Harold Asp, QA Manager

While the information and recommendations set forth herein are believed to be accurate as of the date hereof, ESA, Inc. makes no warranty with respect thereto and disclaims all liability from reliance thereon.

MSDS-041
Rev. A

Appendix E Mass Spectrometer Decoupling Instructions



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Glossary

This glossary provides definitions of a broad variety of terms that are commonly used in HPLC with electrochemical detection. For further information, the reader is referred to a standard text in HPLC such as:

> L.R. Snyder and J.J. Kirkland, "Introduction to Modern Liquid Chromatography", second edition, Wiley Interscience, John Wiley and Sons, Inc., New York, 1979.

or a standard text in electrochemistry such as:

> A.J. Bard and L.R. Faulkner, "Electrochemical Methods", John Wiley and Sons, Inc., New York, 1980.

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a **(Separation factor)**: A measure of the difference in retention of two compounds that takes into account the void volume of a column.

$a = k_2'/k_1' = (V_2-V_0)/(V_1-V_0)$
where: k_1' and k_2' are the capacity factors for peaks 1 and 2 respectively
V_1 and V_2 are the retention volumes for peaks 1 and 2 respectively
V_0 is the void volume for the column

Acquisition Delay: In pulse mode, a delay can be programmed between the time when the analytical voltage is applied to the working electrode and the onset of data acquisition. The purpose of the acquisition delay is to dissipate the current resulting from the charging of the working electrode.

Ampere: The amount of current that passes through a resistance of 1 ohm when a potential of 1 volt is applied. It is equivalent to 1 coulomb/second (abbreviated A).

Amperometry: The measurement of the concentration of an analyte by measuring the electric current generated using a fixed potential. The current is proportional to the concentration of the compound of interest. A series of standards are used to obtain the working curve or the internal standards technique can be used.

Analytical Potential: The potential that is used to effect the desired oxidation or reduction.

Anion: A negatively charged ion.

Anode: The electrode where oxidation (loss of electrons) occurs. The process is shown below for an anion:

$$A^- \rightarrow A + e^-$$

Analyte: The ion or compound that is being determined.

Applied Potential: The potential applied between the working electrode and the reference electrode in an electrolytic cell.

Artifact: An unwanted peak in a chromatogram. It may be due to sample, mobile phase or instrumental contaminants.

Attogram: A unit of mass equal to 10^{-18} gram (abbreviated ag).

Attomole: Amount of material equal to 10^{-18} moles (abbreviated amole).

AUFS: Absorbance units full scale (refers to the full scale deflection of a meter or a pen on a chart recorder; used in conjunction with an absorbance detector).

Autozero: An instrument function, which allows the operator to establish the zero of an output (recorder, integrator, etc.) so that all chromatograms start at the same baseline.

Auxiliary Electrode: The electrode where the complementary reaction to the desired process is taking place. For example, if the working electrode is performing an oxidation, the complementary reaction is a reduction that is occurring at the auxiliary reaction (also known as the counter electrode).

Background Current: The current observed when the mobile phase passing through the electrochemical cell does not contain any electroactive species of interest. The background current may be due to trace materials (contaminants) in the mobile phase or to some component of the mobile phase that is electroactive.

Background Noise: The recorder (or integrator) signal that is observed when the mobile phase passing through the detector does not contain any electroactive species of interest. Variations in the background noise may arise from a number of sources such as the pump, column or detector.

Band: A zone on an HPLC column that contains one (or more) component(s) of a mixture that is being separated.

Band Broadening: The phenomenon of diffusion of a band into a greater volume (e.g., post column band broadening occurs in the tubing between the end of the column and the detector).

Band Spreading: See band broadening.

Baseline Resolution: Chromatographic separation of two compounds such that the peaks are totally isolated from each other. The tail from the first peak intersects the baseline before the second peak begins.

Baud Rate: The rate of transmission of data through the RS232 communications interface from the detector to the computer (and vice versa).

Bed: A collection of a chromatographic media (usually silica) that is used to separate a mixture. In HPLC this is usually termed the stationary phase.

Bonded Phase: A stationary phase in which a functional group is chemically bound to the stationary phase (e.g., 5µm silica). The functional group can be polar (e.g., CN) or non-polar (e.g., C_{18}).

Calibration Curve: A plot of the detector response vs. concentration of a series of known samples (standards). It is used to determine the concentration of the analyte in unknowns.

Capacitance (C): The ratio of the charge on a pair of electric conductors to the potential between the conductors.

$$C=Q/V$$

where: Q is the charge (coulombs)
V is the potential (volts)

When the current in an electric circuit is changed, energy is required to change the electric and magnetic fields that are associated with the flow of charge. Capacitance is the counter force or reactance that tends to counteract the change in the electric and magnetic fields.

Capacitive Current: The current that flows in a circuit (e.g., to an electrode) due to the capacitance of the circuit (electrode) when a potential is changed.

Capacity Factor: The capacity factor is the ratio of the amount of compound adsorbed on the stationary phase to the total amount of compound in the mobile phase.

The capacity factor for an adsorbed compound on an HPLC column can be determined experimentally by use of the following equation:

$$k' = (V_1-V_0)/V_0$$

where: V_1 is the retention volume of the compound of interest
V_0 is the void volume of the column

Carrier: The mobile phase.

Cation: An ion having a positive charge.

Cathode: The electrode where reduction (gain of electrons) occurs. The process is shown below for a cation:

$$M^{+2} + 2e^- \rightarrow M$$

Cell: See Flow Cell.

Cells On/Off: A user settable function of the Coulochem® III detector. Cells On means that a potential is applied to the electrodes of a cell. Cells Off means that the cell is disconnected from the potentiostat.

Channel (Chromatography): A longitudinal void in the column that causes band spreading. Channeling usually occurs near the column walls.

Channel (Detector): One of the potentiostats or electrodes of the detector. The Coulochem III can use 1 or 2 channels.

Chromatogram: A record of a separation that indicates the detector response as a function of time. The chromatogram indicates the elution of electroactive species from the column.

Chromatography: The separation of compounds in a mixture that is effected by exploiting the relative differences between their adsorbtion on a solid phase and their desorbtion into the mobile phase. Chromatography involves the use of a dynamic equilibrium of the compounds between the stationary phase and the mobile phase.

Collapse: The settling of the packing material in a column that leads to a column void.

Column: A cylinder which is used to contain the stationary phase. The mobile phase is pumped through the column to chromatograph the sample.

Column Void: A portion of the column that is not packed with stationary phase. A column void may be due to settling of the stationary phase or dissolution of the stationary phase by the mobile phase.

Column Volume: The volume within a column that is not occupied by stationary phase.

Conditioning Cell: A cell that is placed before the measuring cell. A potential is applied to the conditioning cell that is sufficient to eliminate a potential interferent from the analytical electrode.

Conditioning Potential: In pulse mode, the potential of the electrode is made significantly more positive than the analytical potential and then made significantly more negative than the analytical potential. These conditioning potentials are applied for a short period of time during the analytical process to condition the electrode surface.

Conductance: A measure of the ability of a compound to pass electrical current. The unit of measurement of conductance is the Siemen. Conductance is the reciprocal of the electrical resistance.

Conductometric Detection: To measure the concentration of the analyte by measuring the change in resistance of the mobile phase. This technique will respond to all ions that are present in the mobile phase.

Coulomb: The quantity of charge transferred by a current of 1 ampere for a period of one second.

Coulometric Efficiency: Operation of an electrochemical detector in a manner such that a very high percentage (asymptotically approaching 100%) of the electroactive species of interest is converted (oxidized or reduced) during the detection process.

Coulometry: The measurement of the absolute charge that is used to reduce or oxidize an electroactive species. In coulometry, the integrated current (or charge) is measured; this is proportional to the amount of the compound of interest in the sample via Faraday's law.

Counter Electrode: See Auxiliary Electrode.

Counter Ion: An ion of opposite charge than that of the ion(s) of interest. It maintains electrical neutrality of the solution and column. For example, in the separation of a number of anions, the counter ion might be Na^+.

Current: A flow of electric charge, usually measured in amperes or amps (A, mA, µA, nA, pA, etc.).

Current Range: A setting on the Coulochem III that relates to the current scale used for the signal output. For example a Current Range of 100 nA indicates that a signal from –100 nA to 100 nA can be presented as a signal on the output of the detector for display and/or quantification. Signals higher than 100 nA will be "clipped" or "cutoff".

Current/Voltage Curve: See Voltammogram.

Cyclic Voltammogram: A plot of the electrode current vs. applied potential that is obtained as the potential is changed via a programmed series such that the potential eventually returns to the starting point. A cyclic voltammogram is a complete record of the electroactivity of a compound (ion).

DC Mode: A mode of operation of the electrochemical detector. The potential across the electrodes is maintained at a constant value at all times.

Default Setting: A setting of the detector parameter that is preselected by the manufacturer (e.g., the default filter response is 2 sec).

Degassing: The removal of dissolved gases from the mobile phase. Degassing may be effected by helium sparging or vacuum filtration.

Degree of Dissociation: Fraction of a weak acid and/or weak base that is present in the ionized form in solution.

Detection Limit: The smallest amount of a compound that will produce an observable signal above the background noise. The signal-to-noise ratio that is selected is an integral part of the definition of the detection limit for a given situation (e.g., a system has a detection limit of 2µg of tricyclyne with a S/N of 3).

Detector: A device that is used to determine the presence or absence of analytes in the mobile phase. A detector will provide an electric signal that can be related to the concentration of the compound flowing through the flow cell.

Diffusion: The movement of a compound through a phase under the influence of a concentration gradient. In the column, the compounds of interest will diffuse between the mobile phase and the stationary phase as a part of the separation process. In the immediate area of the electrodes, the ions will diffuse through the eluent to and from the actual surface of the electrode. The rate of diffusion of the ions in the vicinity of the electrode is a critical issue with regard to the limiting current that is observed.

Distribution Coefficient (D): For a chromatographic band that is equilibrated, the distribution coefficient is the ratio of the concentration of the compound on the stationary phase (S_s) to the concentration of the compound in the mobile phase (S_m).

$$D = [S_s]/[S_m]$$

where: $[S_s]$ is the concentration of the compound in the stationary phase.
$[S_m]$ is the concentration of the compound in the mobile phase.

Dry Packing: A technique used to pack a chromatography column. A small amount of the chromatographic support is added to the column and the assembly is vibrated to allow the particles to assume a dense configuration. The procedure is repeated successively until the column assembly is full. Dry packing is used predominantly for large packing materials (e.g., pellicular materials).

E: Abbreviation for Potential (in V or mV).

Efficiency (Chromatographic): A measure of the "effectiveness" of a column for a given separation. Efficiency is measured by the number of theoretical plates on the column.

Efficiency (Detector): The detector efficiency is the ratio of the amount of analyte actually detected to the total amount of analyte that passes through the flow cell.

Electroactive (Species): A compound, element or ion that can be oxidized or reduced at the potential that is being employed by the detector.

Electrochemical Detection: The measurement of an electrical signal that arises from the oxidation or reduction of an analyte in order to determine the concentration of the analyte.

Electrochemistry: The study of the effect of an electrical potential on chemical entities.

Electrode: A device which can take part in an electrochemical reaction by either accepting or donating electrons and thus effecting an oxidation or a reduction, respectively.

Electrolyte: A compound that will conduct electrical current when it is dissolved in a liquid. The strength of the solution will be dependent on the degree of dissociation of the electrolyte into its component ions.

Eluent: The liquid used as a mobile phase in high performance liquid chromatography.

Eluate: The mobile phase as it leaves the column.

Elute: To remove a band from an LC column through continuous flow of the mobile phase.

Elution Volume: The volume (time x flow rate) of solvent that is required to elute a given component of the sample using a given HPLC system. The time is measured from the instant of sample injection to the time of elution (of the center of the band).

Equilibrium: The condition when a chemical system is at rest. At equilibrium, the rate of the forward reaction and the rate of the reverse reaction are equal, and as a result the concentrations of the reactants and products remain constant.

Event Marker: A short pulse that is sent to the recorder (or other output device) that applies a momentary 5% "signal". It is used to denote some action on the output trace (e.g., an event marker might be used to indicate the injection of the sample via a momentary baseline deflection).

Faraday's Law: The relationship that equates the total charge transferred in an electrochemical reaction to the number of equivalents of the reactant.

$$Q = (n)(F)(eq)$$

where: Q is the total charge transferred (coulombs).
n is number of electrons transferred in the electrochemical reaction (e.g., 1 for the Fe(II)/Fe(III) redox couple).
eq is the number of equivalents of reactant.
F is Faraday's constant, 9.64846×10^4 coulombs/equivalent.

Femtogram: A unit of mass equal to 10^{-15} grams (abbreviated fg).

Femtomole: Amount of material equal to 10^{-15} mole (abbreviated fmole).

Filter (Signal): An electronic device that removes a portion of the noise in the signal output. A filter provides a smoother baseline making it easier for peak detection and quantification.

Flow Cell: The part of a detector through which the eluent flows. The analyte is detected by some means such as by oxidation (or reduction) at a working electrode.

Flow Rate: The rate at which the mobile phase is pumped through a column (usually in mL/min).

Fronting: The phenomenon observed when the front of a peak is less steep than the tail of a peak (an asymmetric peak).

Gain: The ratio of the increase in the size of the output signal over the input signal of a detector.

Ghosting: The phenomenon observed when a late eluting peak in one chromatogram is carried over into a second chromatogram.

Gradient: Refers to a programmed change in the composition of the mobile phase during the separation.

Guard Cell: An electrochemical cell that is placed before the injector to aid in the removal of electroactive species from the mobile phase before they can be detected in the analytical cell.

H: Height equivalent to a theoretical plate (see theoretical plate).

Half Reaction: The description of one of the two-electrochemical processes that takes place in a cell. A half reaction can be an oxidation or a reduction (e.g., hydroquinone to quinone or vice versa).

Halfwave Potential: The potential at which the current observed is half of the limiting or plateau current.

HETP: Height equivalent to a theoretical plate (see below).

Height Equivalent to a Theoretical Plate (H): A measure of the efficiency of a chromatographic column.

$$H = L/N$$

where: L is the length of the column.
N is the number of theoretical plates.

Hydrodynamic Voltammogram: A plot of peak current generated or peak area vs. applied potential that is obtained from a series of experiments where the peak current or peak area of a compound is measured at varying potentials. A hydrodynamic voltammogram is used to ascertain the optimal operating potential for electrochemical detection.

i: Abbreviation for current (in A, mA, uA, nA, etc.).

Injector: A device that is used to introduce a specified amount of sample into a LC system.

Interferent: A species that will produce an incorrectly higher signal for the compound of interest due to the fact that it elutes at the same time as the compound of interest and has a detector response that is covered by the conditions used for the compound of interest.

Integrator: A device that can calculate the area under and/or the height of a chromatographic peak. An integrator is used for quantitative analysis.

Ion Chromatography: A separation procedure in which a series of ions are separated (e.g., a series of cations or a series of anions) by use of an ionic column packing material.

Ionic Mobility: The ratio of the average drift velocity of an ion in a liquid or a gas, relative to the electric field. Ionic mobility affects ionic conductance. Ion mobility is quite temperature dependent (approximately 2%/°C).

Ionization Constant (K): Equilibrium constant for the dissociation of a weakly ionized compound. For the ionization:

$$AB \rightarrow A^+ + B^-$$

the ionization constant is:

$$K = [A^+]\,[B^-]/\,[AB]$$

where: $[A^+]$, $[B^-]$ and [AB] are the concentrations of the three species in solution.

Ionization constants are frequently expressed in a logarithmic basis as pK, where:

$$pK = -\log K$$

Isocratic: Refers to the use of a mobile phase with a constant composition during the chromatographic separation.

Limiting Current: The maximum observed current in an electrochemical cell, especially when performing a hydrodynamic voltammogram or current/voltage curve.

Linearity: Refers to the ability of a property of a sample (e.g., concentration) to be directly related to an experimentally measurable parameter (e.g., current) in a proportional relationship. The linearity of a calibration curve is a factor of critical interest.

Linear Velocity: Average speed of the solvent front though a column (usually measured in cm/sec).

Longitudinal Diffusion: Band spreading in the longitudinal direction of a column as a result of randomized or eddy diffusion of the compound of interest.

Mass Transfer: Transfer of mass from the mobile phase to the stationary phase (or vice versa) or from the mobile phase to an electrode (or vice versa). Stationary phase mass transfer thus refers to the diffusion of molecules into and out of the stationary phase.

Method: A set of analytical parameters that defines the operation of the Coulochem III detector.

Microampere: A unit of current equal to 10^{-6} amps (abbreviated μA).

Microgram: A unit of mass equal to 10^{-6} gram (abbreviated μg).

Micromole: Amount of material equal to 10^{-6} mole (abbreviated μmole).

Milliampere: A unit of current equal to 10^{-3} amps (abbreviated mA).

Milligram: A unit of mass equal to 10^{-3} grams (abbreviated mg).

Millimole: Amount of material equal to 10^{-3} moles (abbreviated mmole).

Millivolt: A unit of potential equal to 10^{-3} volts (abbreviated mV).

Mobile Phase: The liquid that is used to transport a sample mixture through a chromatographic bed. Often referred to as the eluent.

Mode: A type of operation provided by the Coulochem III electrochemical detector (e.g., DC mode, SCAN mode and PULSE mode). In addition, the Mode key accesses the system menu.

N: Number of theoretical plates for a column as measured by the efficiency of a column. N can be calculated by the equation:

$$N = 16 (t_R/t_W)^2$$

where: t_R is the retention time (in seconds).
t_W is the baseline width of the peak (in seconds).

n: Number of electrons transferred per molecule or ion in a reduction or oxidation.

Nanoampere: A unit of current equal to 10^{-9} ampere (abbreviated nA).

Nanogram: A unit of mass equal to 10^{-9} gram (abbreviated ng).

Nanomole: A unit of mass equal to 10^{-9} mole (abbreviated nmole).

Nernst Equation: The relationship that describes the potential of a reversible half reaction as a function of the concentration of the oxidized and reduced form of the electroactive species.

$$E_{app} = E^{\circ\prime} - (0.059/n) \log [OX]/[RED]$$

where: E_{app} is the applied potential (in Volts).
$E^{\circ\prime}$ is the potential using the standard conditions (25°C, 1 atm).
[OX] and [RED] are the concentrations of the oxidized and reduced forms of the redox couple (in moles/liter).
n is the number of electrons transferred in the reaction.

Noise: The signal that appears on a chromatographic output due to a variety of events that are not a desired response of the detector. Noise can be random or regular (e.g., due to pump pulsations).

Offset: The deflection of a recorder trace baseline so that two (or more) chromatograms can be overlaid on a single presentation. Also, the moving of a recorder trace baseline so that the output range of the detector/recording device can be altered (e.g., to display for negative dips from the baseline).

Ohm's Law: The fundamental relationship governing the potential, current and resistance of an electrical circuit.

$$E = iR$$

where: E is the potential between two points in a circuit (in Volts).
R is the resistance between the two points (in Ohms).
i is the observed current (in Amperes).

Organic Modifier: An organic solvent (e.g., methanol and acetonitrile) that is used to alter the elution characteristics of an aqueous solution that is used as the mobile phase.

Output: The signal that the detector provides to the recorder or other device (e.g., a data station).

Oxidize: The process of removing an electron (or more than one electron) from an atom, ion or compound.

Oxidizing Agent: An element, ion or compound that is capable of oxidizing another species by accepting an electron (or more than one electron).

Packing: The stationary phase for HPLC.

Parallel Port: The output device for the printer. In a parallel port, several lines are used on a simultaneous basis to transmit one data point or item of information.

Parity: A method of providing a mechanism for the validity of the transmission of data via an RS232 interface.

Particle Size: The average particle diameter of the column packing material (usually in microns (μm)).

Parts Per Billion: A unit of concentration equal to nanograms/gram (10^{-9}). Usually abbreviated ppb.

Parts Per Million: A unit of concentration equal to micrograms/gram (10^{-6}). Usually abbreviated ppm.

Parts Per Trillion: A unit of concentration equal to picograms/gram (10^{-12}). Usually abbreviated ppt.

Passivation: Preparation of the HPLC system to remove electroactive materials and to prevent further electroactive materials from entering the mobile phase.

Peak: An indication on the chromatographic output that the electrochemical detector has observed the presence of an electroactive compound in the mobile phase. The pen response will be above the baseline. A peak has an approximately Gaussian shape.

Peak Area: The area underneath a recorder trace and above the extrapolated baseline. The peak area is proportional to the amount of material in the sample. For an electrochemically-generated peak, the peak area can be related to the charge of the reaction that is related to the amount of the analyte by Faraday's Law. The peak area can be approximated by the equation:

$$A = bh$$

where: b is the width of the peak at the base.
h is the maximum peak height.

Peak Height: The measurement of the distance on a chromatogram from the baseline to its highest point for a peak. For an electrochemically generated peak, the peak height is usually given in terms of current (amps).

Peak Width: The breadth of the peak. The peak width is frequently measured at the intensity that is equivalent to 0.5 of the peak maxima. The peak width is usually expressed in minutes or seconds.

Picoamp: A unit of current equal to 10^{-12} amps (abbreviated pA).

Picogram: A unit of mass that is equal to 10^{-12} grams (abbreviated pg).

Picomole: Amount of material that is equal to 10^{-12} mole (abbreviated pmole).

pK: See Ionization Constant.

Plate: In distillation theory, a plate is the height required for one vaporization and one condensation process. It is a measure of the efficiency of the column. This concept has been transported to HPLC, where it is a measure of the efficiency of the column.

Potential: The relative voltage or electroactive force between two electrodes (usually measured or applied between the working electrode and the reference electrode) and is measured in volts.

Potentiostat: An electronic assembly that interfaces the electrodes of a cell to the operating system of the Coulochem III. Several potentiostats can be placed in the Coulochem III detector unit. The potentiostat is responsible for applying and maintaining a given potential between the reference and working electrodes by passing current through the working and counter electrodes.

Pulse Amperometry: An analytical technique in which the potential applied to the working electrode is changed during the analysis. Pulses of high positive and high negative potentials are applied to the electrode to condition it for the next analysis.

Pulse Mode: See Pulse Amperometry.

Redox Couple: The oxidized and reduced forms of a chemical species (e.g., Fe (II) and Fe(III)).

R: Abbreviation for current range (in A, mA, µA, nA, etc.).

Reduce: The process of gaining one or more electrons by an ion, element or compound.

Reducing Agent: A compound, element or ion that is capable of reducing another species by donating one or more electrons.

Reference Electrode: In an electrochemical cell, the reference electrode is used to provide a stable potential from which the potential of the working electrode is measured.

Remote Communication: The mode of operation in which the control of the detector is via an external device (e.g., a personal computer).

Resin Based Column: A chromatographic column, which employs a polymeric resin. The functional groups are chemical derivatives of the polymer itself. This is in opposition to silica columns, where the functional groups are chemically bonded to the silica.

Resistance: Opposition offered by a component to the flow of current in an electrical circuit.

Resolution: The separation of two peaks in a chromatogram. The resolution R is defined by:

$$R = (V_2-V_1)/((0.5)(W_2-W_1))$$

where: V_1 and V_2 are the retention times for peak 1 and peak 2.
W_1 and W_2 are the baseline width of peak 1 and peak 2.

Response Factor: The amount of a compound that was injected, divided by the area under the peak.

Retention Time: The time that has elapsed between the injection of a sample and the time when the detector response is maximized (i.e., the top of the peak) for a given compound.

Retention Volume: The volume of mobile phase that is required to elute a compound from a column.

Reverse Phase Column: A column in which the stationary phase is less polar than the mobile phase used with it. Typically a reverse phase column consists of a column packing material with either an alkyl chain (e.g., C_{18}) or a somewhat polar side chain (e.g., CN).

Reversible Reaction: An electrochemical reaction in which the oxidized form of a species and the reduced form can be readily converted into each other. In the region of the standard electrode potential for the process, a small change in the potential can result in a change in the direction of the electrochemical reaction.

RS232: A standard interface scheme for the transmission of digital information between a computer and a detector. This is commonly called the serial mode of data transmission.

Scan Mode: Operation of the detector in such a way that the operating potential is changing as a function of time. The potential will start at a fixed value, go through two limits and then to a final potential. Scan mode is generally used to obtain a complete description of the current-voltage phenomenon for the compound by obtaining a cyclic voltammogram.

Screen Mode: Use of an analytical cell to remove (screen) electroactive impurities or unwanted analytes from the mobile phase.

Selectivity (Chromatographic): The ability of a chromatographic system to separate two compounds. Selectivity is measured in terms of the resolution of the chromatogram.

Selectivity (Detector): The ability of a detector to provide a distinct signal for the desired compound in a mixture, while discriminating against all other materials in the mixture.

Sensitivity: Refers to the minimum detectable quantity of an analyte with a given set of analytical conditions. The signal-to-noise ratio of the measurement should be indicated.

Serial Port: A communication port on the Coulochem III that uses the RS232 protocol to transmit information. A method of transmission of data in which information is transmitted one bit at a time.

Signal-to-Noise Ratio (S/N): The signal-to-noise ratio for a data point is obtained by dividing the signal by the noise level that is associated with the measurement. A larger S/N ratio is more desirable than a small one. As the S/N ratio approaches unity, the level of certainty that a peak is real falls.

Signal Output: See Output.

Slurry Packing: A method of packing an HPLC column whereby the particles are suspended in a solvent and forced into a column by pressure (as opposed to dry packing). Slurry packing is commonly used to pack small particles (e.g., below 10µm diameter).

Solvation: The process of dissolving a material by a solvent.

Solute: The substance that is dissolved in a solvent.

Solvent: A liquid that is used as the mobile phase in HPLC or the liquid that dissolves the solute.

Sparge: The process of bubbling He or N_2 through a filtered solvent. Sparging is used to displace dissolved gases that have a deleterious effect on the separation and/or detection of analytes of interest. Sparging serves to displace CO_2, which could change the pH of a buffered solvent, and O_2, which could take part in electrochemical processes.

Standard: A solution containing a known amount of the compound of interest. The detector response is measured for a series of standards to obtain a standard curve.

Stationary Phase: The column packing material where adsorbtion and desorbtion of the components of the sample takes place. The mechanism of the interaction could be via ion exchange, adsorbtion or size exclusion.

System Menus: A selection of instrument parameters for the Coulochem III detector that are infrequently changed.

Tailing: In theory, a chromatographic peak is Gaussian. If the longer retention time profile of the peak is not Gaussian, the phenomenon is termed tailing and the peak is termed asymmetric.

Target Electrode: The working electrode for the Model 5040 and 5041 cells.

Temperature Coefficient: The rate of change of a phenomenon that is related to a change in the temperature.

Test Electrode: See working electrode.

Theoretical Plate: See Plate.

Time Constant: The period of time it takes for the signal to drop to 2% of the signal after the stimulus is removed.

Timeline: A mode in the Coulochem III detector that can cause events (such as a gain change, autozero, event mark, etc.) to occur at designated times during a chromatogram.

Van Deempter Plot: A plot of the height equivalent to a theoretical plate as a function of the linear velocity of the mobile phase. A van Deempter plot is used to determine the linear velocity that provides the most efficient separation (i.e., the shortest HETP).

Void: A part of an HPLC column that does not contain packing material. A large void causes a dramatic decrease in the efficiency of a column.

Void Volume: The available volume of an LC system between the injector and the detector. The void volume is the total volume of the system, less the volume occupied by the column packing. This term is approximated by the peak in the chromatogram that corresponds to the unretained analytes.

Volt: The unit of electrical potential difference.

Voltammogram: A plot of the current from an electrochemical system as a function of the potential that is impressed upon it. See also Hydrodynamic Voltammogram and Cyclic Voltammogram.

W: Peak width or band width.

Wall Effect: Band spreading as a result of solvent flow along the column wall being different than the flow through the center of the bed.

Word: The basic unit of information that is transmitted by the serial interface. The length of the word is either 7 or 8 bits.

Working Electrode: The electrode where the desired electrochemical process is occurring.

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